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ENVIRONMENTAL ANALYSIS

TECHNIQUES,
APPLICATIONS AND
QUALITY ASSURANCE

Edited by

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PREFACE

This book treats three different aspects of environmental analysis: the use of various analytical techniques, the applications to trace analysis of pollutants, mainly organic compounds, and the quality assurance aspects, including the use of certified reference materials for quality control of the whole analytical process. The need for more selective and sensitive analytical methods to help solve the numerous trace analysis problems in complex environmental samples is undisputed. The tremendous growth of environmental analysis in the last few years is due to the assessment of the quality of our environment being dependent on the availability of reliable analytical data as scientific conclusions and political decisions on environmental issues are based on their interpretation. Consequently, the development of analytical methods for pollutants has gone hand in hand with the adoption of regulations controlling their release into the environment.

One of the key elements of effective quality assurance is the availability of analytical techniques which can generate reliable data and their appropriate application. Obviously, the number and variety of these techniques currently in use in environmental analysis is so vast that their complete coverage in one book is impossible.

The book is organized in four sections. The first describes field sampling techniques and sample preparation in environmental matrices: air, water, sediment and biota. It provides a critical review of air monitoring sampling, the development of solid-phase extraction principles including on-line precolumn technologies for trace enrichment of organics in water, the sample handling of volatile organic compounds and the various aspects of the extraction and clean-up procedures for the more persistent organic compounds in sediment and biota matrices.

The second section covers the application of robust analytical techniques to the characterization of environmental target compounds included in many priority lists. The recent developments in the analysis of PCB congeners are discussed and the different official methods of analysis of pesticides in water using gas chromatography are reviewed. Various liquid chromatographic techniques, including coupled columns and pre- and post-column derivatization, used for the determination of phenolic compounds, polar pesticides and mycotoxins and phycotoxins, respectively, are covered. The final chapter in this section covers the use of liquid scintillation and radiochemical analyses for the determination of radionuclides in environmental samples.

Validation and quality assurance is one of the key parameters in all analytical measurements. These aspects are described in the third section of the book, and comprise a general discussion about quality assurance and the use and preparation of certified reference materials in environmental analysis. This is a very important issue since without quality assurance, questionable data can be obtained which can result in questionable conclusions.

The last section of the book deals with the newest techniques applied to the field of environmental analysis. Shpol'skii and synchronous fluorescence spectroscopy, and mass spectrometric approaches including the use of desorption ionization methods such as fast atom bombardment, are described for the characterization of PAHs and detergents, re-

spectively. The development of different interfacing systems in LC-MS, hyphenated techniques and various CE approaches such as CZE, electrokinetic capillary and isotachopheresis in environmental analysis, are also considered.. Although some of these techniques are not widely used at present, with only a few specialized laboratories currently using them on a routine basis, their inclusion in the book is to give some future perspectives to this continuously changing field.

By the nature of its content and written as it is by experienced practitioners, the book is intended to serve both as a general reference for post-graduate students as well as a practical reference for environmental chemists who need to use the analytical techniques for environmental studies and analytical chemists needing information on the complexity of environmental sample matrices and interferences. Each chapter includes sufficient references to the literature to serve as a valuable starting point for a more detailed investigation. The broad spectrum of the book, with its description of novel techniques, its many applications to the analysis of a variety of compounds, including applications to the analysis of metabolites from pesticides, PAHs and detergents, and the focus on quality assurance, makes it unique in many ways.

I thank the authors for their time and effort in preparing their chapters. Without their cooperation and engagement this volume would certainly not have been possible.

Barcelona, May 5, 1993

D. Barceló

Chapter 1

Sampling techniques for air pollutants

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1.1. INTRODUCTION

Monitoring of air constituents has become an important task for many scientists. Whether they are interested in meteorology, atmospheric physics, or chemistry, all have the same problem: "How can I describe the air compartment in the right way"?

Air constituents include not only gas molecules, but also finely dispersed materials of a homogeneous or heterogeneous nature. The problem of describing air exhaustively using physical or chemical techniques is often limited by two properties of the aerosols: first, they consist of multi-phase particle systems (such as the model aerosol particles shown in Fig. 1.1) and, secondly, the mass of a typical particle encountered in such an aerosol is in the ultratrace range (for example, the mass of one 100-nm particle is estimated to be of the order of femtograms).

Considering the fact that each particle has a different microstructure, adlayers, etc., one can imagine the impossibility of exhaustively describing air. So, in general, we measure only the averaged properties of a highly dynamic particle distribution and gas mixture.

From the analytical point of view, this means that we have to apply analytical techniques of the highest sensitivity and selectivity. To deal with the inherent dynamics of the system an on-line capability is also imperative. At present, for most problems this is still nearly impossible. Table 1.1 lists the most relevant analytes presently of interest in emission monitoring, together with the necessary detection limits. The figures originate from various governmental clean air acts. Of course the list is incomplete but it is intended to represent those analytes which are most relevant. For immission monitoring, a much lower detection limit (up to a factor of 10 000) has to be achieved. These limits can only be achieved by using a considerable enrichment prior to the intended analytical technique.

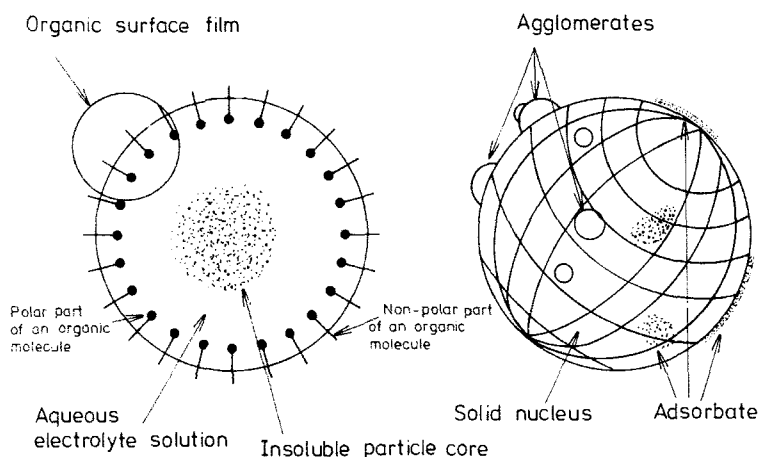


Fig. 1.1. Possible microstructure of particles.

The problem to be discussed in this chapter is how one can transfer the components of interest, without changes in the physical and chemical states, to the analytical process. This means that any artifact formation during sampling has to be avoided.

In order to evaluate the different possible strategies, we divide sampling techniques as follows:

- sampling without suction, with deposition;
- sampling with suction, without deposition;
- sampling with suction, with deposition;
 - (a) discontinuous sampling;
 - (b) continuous sampling.

We discuss existing sampling principles that may be suitable for an application in the above-mentioned sense, but also cover new potential solutions and developments. The discussion concentrates mainly on particle-related analytical techniques.

1.2. SAMPLING WITHOUT SUCTION, WITH DEPOSITION

The typical method of “passive sampling” is the accumulation of material towards a natural or artificial sink. Very commonly in gas sampling one uses diffusion tubes with coated inner walls (e.g. using Tenax for organic vapors [1]). Other well known sampling techniques for air monitoring use more or less open coated beakers or adhesive tape samplers [2]. The same principle is used for fog sampling, in mimicry of a spider’s web [3].

It is obvious that this technique cannot be used for concentration measurements. The samplers generally produce a temporally and spatially unknown mass flux. Consequently, the deposited mass of substances reflects only the quality of such an artificial or natural sink and the forces acting on the air constituents during the sampling period.

Forces that remove particles and gases from an air volume are condensation, impaction, diffusion, thermophoresis and diffusiophoresis. Other mechanisms are sedimentation and turbulent transfer. The properties of the receptor and the removed substances (reactivity, humidity, solubility, size, charge, roughness, alkalinity, etc.) will determine the deposition efficiency achieved. These facts make a theoretical treatment extremely difficult. We have to conclude that all published and recommended sampling techniques based on passive sampling will produce results that are valid only for the special type of collector and receiver used. The results will be only comparable to those from other collectors in very favorable circumstances. The same holds for other types of concentration measurements.

A very interesting new sampling scheme, presented by Steen [4], was based on a simultaneous, isokinetic sampling of both particulate and gaseous air constituents. The collector is shown in Fig. 1.2. Isokinetic sampling conditions (which means that there is no particle separation caused by different sampling velocities around the inlet and inside the duct of a sampler) are established by the use of Venturi nozzles and a weather-vane suspension for the collector tube. During sampling, the axis of the cylinder is oriented parallel to the flow lines and the aerosol is sucked in isokinetically through the inlet nozzle. A coaxially fixed field emission electrode, held at a high electrical potential at the inlet puts a unipolar charge on all particles. The meteorological conditions (wind speed

TABLE 1.1

TARGET SUBSTANCES OF INTEREST IN AIR MONITORING (EMISSION SITUATION)

Most interesting analytes		Necessary detection limit
Gas phase	Hydrogen chloride	< 60 ppb
	Nitric acid	< 10 ppm
	Hydrogen sulfide	< 10 ppm
	Sulfur trioxide	< 50 ppb
	Mercury	< 10 ppb
	Benzene, toluene, xylene	<100 ppb
	Nicotine	< 70 ppb
	Organic aromatic amines	No threshold limit recommended
Aerosol phase	Sulfuric acid	< 50 $\mu\text{g m}^{-3}$
	Diesel exhaust	No threshold limit recommended
	Asbestos fibers	No threshold limit recommended
	Polycyclic aromatic hydrocarbons	No threshold limit recommended
	Nickel	No threshold limit recommended
	Polychlorinated biphenyls	<500 $\mu\text{g m}^{-3}$
	Polychlorinated dibenzodioxins and dibenzofurans	<100 pg m^{-3}
	Chromium trioxide	<100 $\mu\text{g m}^{-3}$
	Cadmium	< 40 ng m^{-3}
	Zinc	<100 $\mu\text{g m}^{-3}$

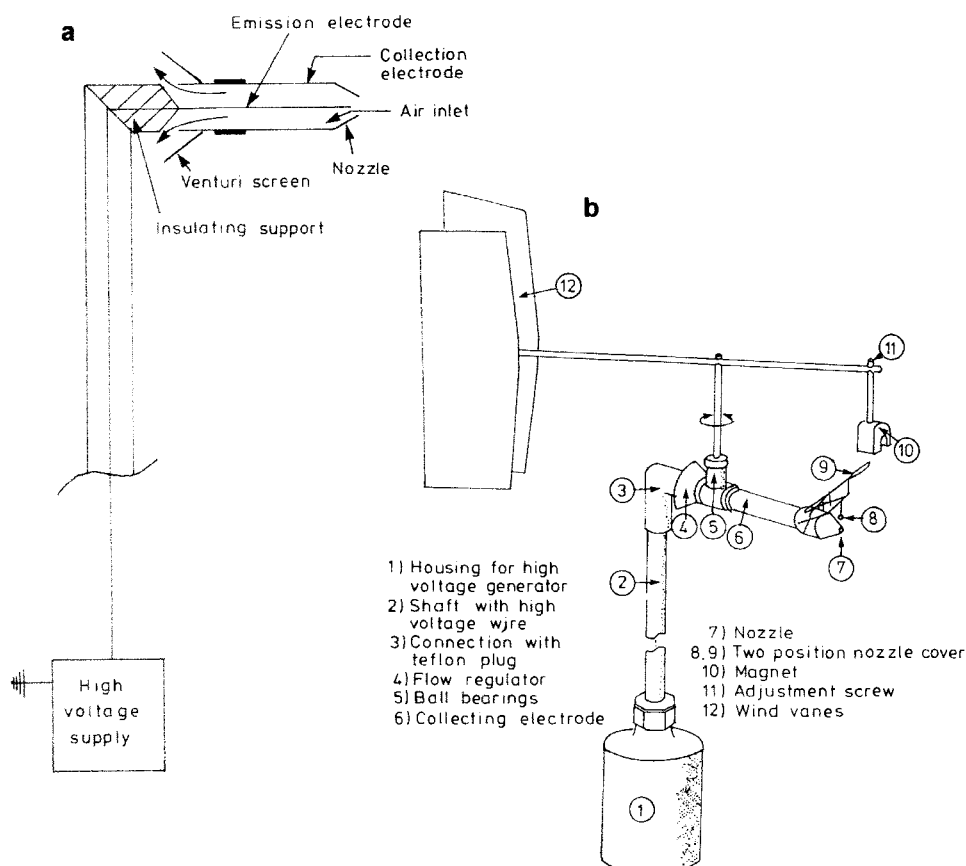


Fig. 1.2. (a) Schematic diagram of the basic isokinetic electrostatic particle sampler. (b) Sampler with wind vane control of nozzle cover for wind-directed measurements.

and wind direction) act as driving forces for the sampling by causing an underpressure across the venturi nozzles. The only limitations result from the varying charging efficiencies for incoming particles of different diameter and the receptor quality of the inner wall. Unfortunately, this system has never become popular, but would seem to be worth developing further.

1.3. SAMPLING WITH SUCTION, WITHOUT DEPOSITION

In this chapter, we recognize the great advantage of this sampling philosophy, because sampling with suction and without deposition is identical to in-situ and on-line sampling. Historically, on-line and in-situ sampling were a response to the increasing demand for fast signal generation and acquisition, which are required by modern control and regulation technologies, e.g. of combustion processes. However, producing a traditional off-line measurement signal for a trace constituent often involves the enrichment of hundreds of

cubic meters of air. This may lead to serious sampling artifacts, since the dispersed state of matter is changed by deposition on a very small surface such as a membrane filter, or by concentration into a very small volume, for example as a condensate. Extensive reaction between all possible reaction partners is a not unexpected result.

Which artifacts are possible? Table 1.2 shows some known artifacts reported during recent years. These artifacts can occur, but are not consequently induced by the sampling process. Whether an artifact is produced during enrichment depends on many variables, and one can only optimize sampling with respect to the analyte of interest. We must keep in mind that the method applied may be the worst solution for another compound.

There is sometimes disagreement about the existence of artifacts, mainly on the assumption that the presumed reactions would have happened earlier, in the atmosphere. This sounds reasonable but holds true only in well-mixed, aged air, and not in populated

TABLE 1.2

POSSIBLE ARTIFACTS DURING SAMPLING OF AIR CONSTITUENTS

Artifact (caused by)	Example
Gaseous air constituents	
Memory effects in transfer lines and gas/particle separation means (adsorption of dipolar molecules)	Adsorption of HNO ₃ , HCl or HF within stainless steel tubing [5], filter holders and membrane filters [6,7]
Production of acidic or alkaline gases (HCl, HNO ₃ , NH ₃) (acid/base reaction within sampled aerosols)	Topochemical interaction of strong acidic aerosol (H ₂ SO ₄ , HSO ₄ ⁻) with already deposited particles (MeNO ₃ , MeCl) [8,9]; catalytic formation on deposited particles (SO _x) [10–18]
Particulate material (aerosols)	
Decomposition of originally solid particulates or liquid droplets (due to temperature change or change of saturation pressure in ambient air with respect to substance of interest)	NH ₄ NO ₃ (l) ⇌ HNO ₃ (g) + NH ₃ (g) [19–25] PAH(adsorbed) ⇌ PAH(g) [26–31]
Interaction of reactive gases with particulate material	2NH ₄ NO ₃ (s) + H ₂ SO ₄ (l) → 2NH ₄ HSO ₄ (l) + 2HNO ₃ (g) [6,8,9] NH ₃ (g) + H ₂ SO ₄ (l) → NH ₄ HSO ₄ (s) [32,33] 2NH ₄ Cl(s) + H ₂ SO ₄ (l) → (NH ₄) ₂ SO ₄ (s) + 2HCl(g) [6,8,9] NO _x + HNO ₃ + PAHs → Nitro-PAHs [34–41]
Change of particle size distribution (due to electrostatic deposition or volatilization)	O ₃ + π-electron rich systems, e.g. PAHs, terpenes [42–45] Strong losses of charged particles in PVC tubing; loss of liquid droplets in slightly heated tubing (e.g. tobacco aerosol)

areas such as cities. In an urban environment, a rapid mixing during the sampling period cannot be expected, due to the presence of a large number of diffuse emission sources (cars, chimneys, factories, etc.) and extremely complicated air streaming conditions. Many problems with distinct odor emissions testify to this. Another well-known example is that acidic aerosols and high concentrations of ammonia were found to co-exist in large cities. Chemically one might not expect this, but one explanation may be that there is a complicated source pattern, for which the different aerosol systems need a considerable time to reach a final thermodynamic equilibrium mixture.

The best way to avoid artifact formation is to use in-situ sampling, where the relevant substance is determined in its natural state of suspension. Such in-situ measurement techniques are at an early stage of development and only a few, but very promising, applications have been reported. The most prominent techniques with their reported detection limits are summarized in Tables 1.3 and 1.4.

Many of the listed techniques are based on laser light sources. One reason for this selection is that the sensor signal (pressure in photo-acoustic spectroscopy, light emission in the case of LIBS, fluorescence and Raman spectroscopy, and light scattering in the case of LIDAR) is proportional to the exciting light intensity. An excellent example of an experimental technique for the in-situ measurement of individual aerosol particles using Raman scattering is given by Fung and Tang [83]. A schematic diagram for the single-particle Raman spectrometer is shown in Fig. 1.3. The particle cell consists of an electrodynamic suspension trap. Two electrodes provide the necessary levitating DC-potential for the charged particle and an AC-potential applied to the ring-electrode keeps the parti-

TABLE 1.3

HIGHLY SENSITIVE IN SITU GAS MONITORING TECHNIQUES

Detection principle	Analyte (detection limit)	References
Light absorption		
Photo-acoustic spectroscopy	Mercury (100 ppt)	[46]
	1,3-Dichloropropane (250 ppb)	[47]
	Ammonia (2 ppb)	[48,49]
Fourier transform infrared spectroscopy	Nitric acid: (4 ppb)	[50]
IR tunable-diode laser spectroscopy	Nitric acid: (100 ppt)	[51]
Light emission		
Fluorescence spectroscopy (laser-induced)	Nitric acid (100 ppt)	[52,53]
	Anthracene (10 ppb)	[54]
Laser-induced breakdown spectroscopy	Chlorine (8 ppm)	[55–58]
Chemiluminescence	Nitrogen dioxide: (5 ppt)	[59]
Physical properties		
Ion mobility	Nitroglycerine (180 ppt)	[60–63]
	Hydrogen chloride (1 ppb)	
Aerosol ionization	Sulfur dioxide (200 ppb)	[64]

TABLE 1.4

HIGHLY SENSITIVE IN SITU AEROSOL MONITORING TECHNIQUES

Detection principle	Analyte (detection limit)
Light absorption	
Photo-acoustic spectroscopy	Carbon or diesel aerosol (300 ng m^{-3}) [65–68]
Optothermally induced light scattering	Sulfate ($5 \mu\text{g m}^{-3}$) [69,70]
Optothermally induced particle charging	Carbon ($<1 \mu\text{g m}^{-3}$) [71,72]
Light emission	
Laser-induced, time-resolved fluorescence	e.g. Perylene ($<10 \text{ ng m}^{-3}$) [73–75]
Laser-induced breakdown spectroscopy	Cadmium ($20 \mu\text{g m}^{-3}$) [76,77]
Analysis by ICP-atomic emission spectroscopy	Cu, Ti, Mg (LOD not given) [78–81]
Analysis by Raman spectroscopy	Sulfate, nitrate (single particle analysis) [82–87]
IR laser back-scattering	Sulfuric acid ($<1 \text{ g m}^{-3}$) [88,89]
Analysis by measurement of electrons, ions or ionized particles	
Photoelectron spectroscopy for chemical analysis	Iodide (LOD not given) [90]
Mass spectrometry	4,4'-Dibromobiphenyl (10^{-13} g abs.) [91,92]
Aerosol photoemission	e.g. Benzo[a]pyrene ($1 \mu\text{g m}^{-3}$) or nickel oxide (250 ng m^{-3}) [93–101]
Analysis by observation of hygroscopic properties	
Aerosol mobility chromatograph	Sulfuric acid (1 ng m^{-3}) [102]
Condensation on monodispersed aerosols	Sulfuric acid ($<1 \mu\text{g m}^{-3}$) [103,104]
Analysis by chemical identification reactions	
Selective stoichiometric reaction and observation of the products	Sulfuric acid ($0.2 \mu\text{g m}^{-3}$) [105] Hydrochloric acid aerosol ($800 \mu\text{g m}^{-3}$) [106]

cle in the center of the cell. Such arrangements open new possibilities for studying the chemical behavior of particulate materials without any artifact formation.

An on-line PAH measurement system based on laser-induced and time-resolved fluorescence is shown in Fig. 1.4. It consists of a fast ($\lambda_p = 300 \text{ ps}$) nitrogen laser as light source and a fast digital storage oscilloscope in addition to connecting quartz fiber optics, monochromator, mirror and beam trap. Preliminary results yielded a detection limit of some $100 \text{ ng pyrene/m}^3$. Work is currently in progress on deconvolution algorithms for the interpretation of superimposed fluorescence decay processes and emission spectra of several PAHs adsorbed on one particle [107]. The use of laser scanners should make a three-dimensional screening of air volumes possible within the near future. This would be of considerable importance for finding the sources of aerosol emission (e.g. in clean rooms or for fire detection).

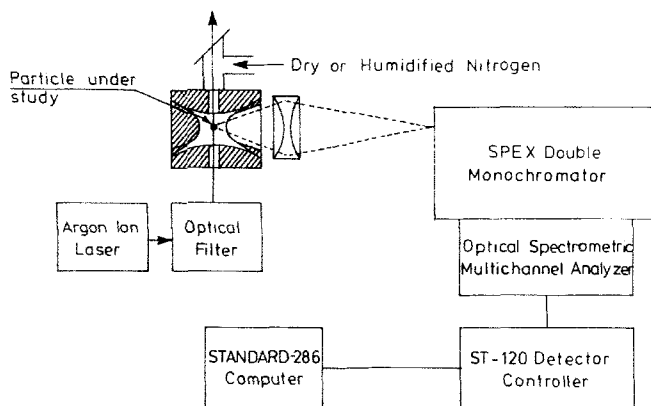


Fig. 1.3. Experimental set-up for single-particle Raman spectroscopy.

Great interest has arisen in light-absorbing particles since a change in the world's climate has been noticed, and there has been speculation about a possible influence of soot particles on the total radiation budget [108]. Up to now, photo-acoustic spectroscopy has offered the only available approach for on-line, in-situ measurement of the absorption of radiation by ultrafine airborne particles. A new detection technique, using a high-power laser working in the infrared spectral region ($I_p \sim 1$ W), is depicted in Fig. 1.5. The advantage of such a laser diode, compared with other high-power lasers in the visible region, is its emission wavelength of 800 nm, the small size of the laser, and the possibil-

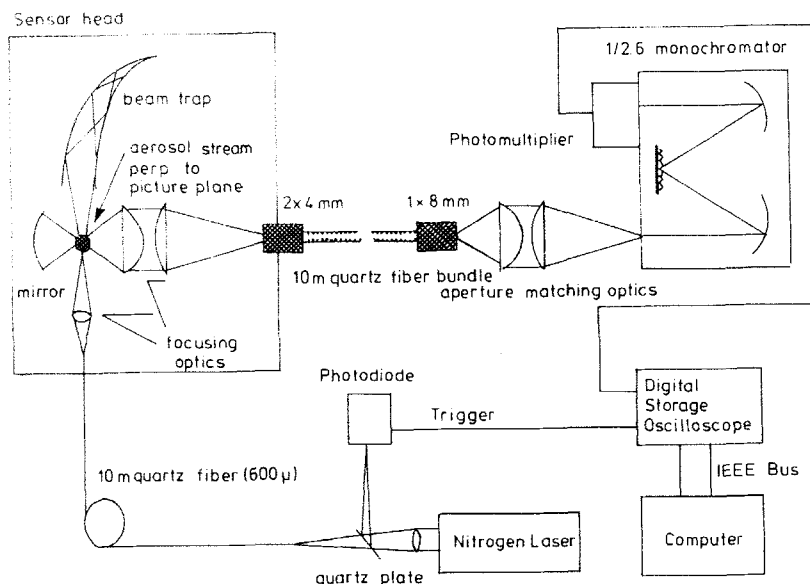


Fig. 1.4. Experimental set-up for on-line aerosol fluorescence detection of particle-bound PAHs.

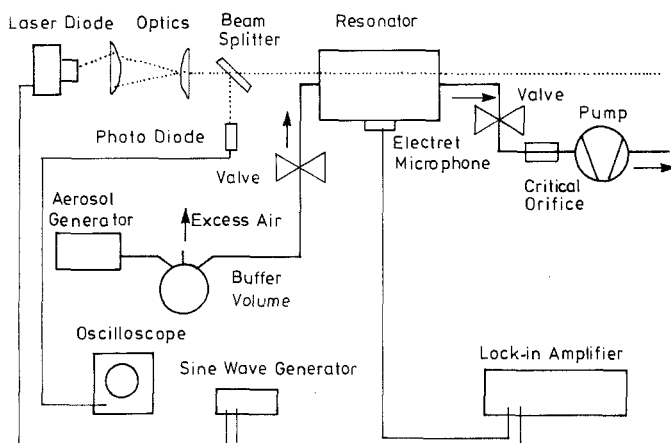


Fig. 1.5. General scheme of experimental set-up for the generation and photo-acoustic detection of carbonaceous aerosol.

ity of electronic intensity modulation of the light instead of modulation by a mechanical chopper. This results in a decreased background signal and an extended modulation frequency range. By using a resonant photo-acoustic cell, a detection limit of $10 \mu\text{g m}^{-3}$ of carbon in a free flowing aerosol stream has been achieved [68].

One special application of the photoelectric aerosol sensor (PAS) as a detector for particle-bound PAHs in diesel exhaust is given in Fig. 1.6. The photoelectric aerosol sensor consists of the detection system, the aerosol sampling head, and the measuring system. With the dilution air controlled by the mass flow controller, 1, in the dilution system, the exhaust aerosol is mixed with particle-free air at the entrance of the sampling head. The system allows dilution ratios up to 1:32. With this dilution system, the PAS can be used to monitor PAHs in an undiluted car exhaust. Within the measuring system, the aerosol is stripped of the charged particles in an electrostatic filter. The neutral particles then enter the illumination section, where a PAH mass-related particle photocharging takes place (at a wavelength of 185 nm). Only surface-enriched molecules with an extended π -electron-system show a high photoelectric quantum yield. The PAS signal was correlated with the PAH-content determined by sampling on a parallel filter and GC determinations. The measurements indicated that the photoelectric signal is influenced by photoelectrically inactive paraffins adsorbed on the diesel particles. In this case, it was possible to correct the PAS signal for the true PAH content. The results shown in Fig. 1.7 are valid for various types of engines and loading conditions.

In our opinion, sampling with suction but without deposition is a most promising analytical tool for the future. A large number of classical optical techniques can be applied to the air constituents, avoiding many possible artifacts. However, there is a major restriction for particulate or particle-bound analytes because only a few of these techniques are really mass-related, most of them being surface-sensitive.

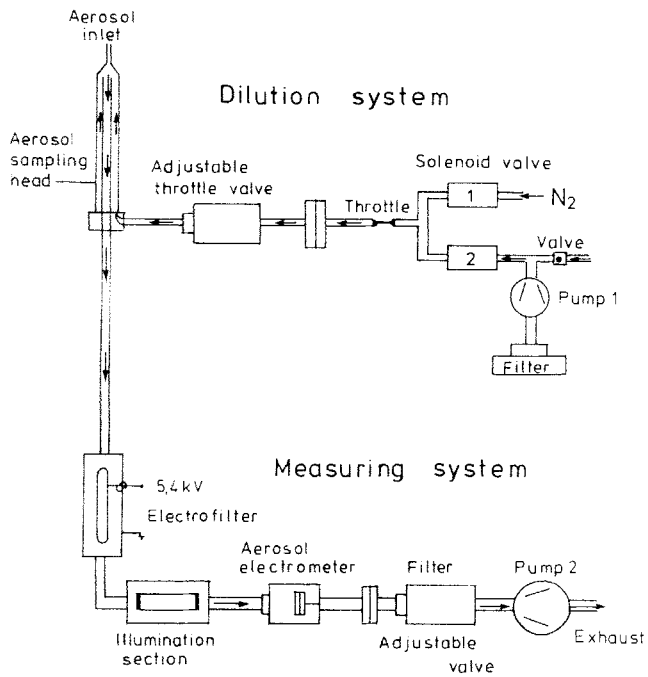


Fig. 1.6. Set-up for on-line detection of particle-bound PAHs in diesel exhaust with the photoelectric aerosol sensor (PAS).

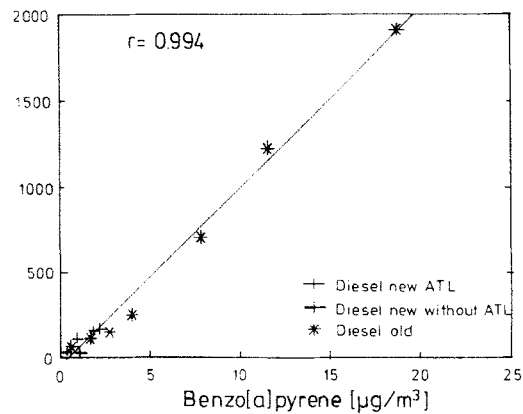


Fig. 1.7. Correlation between PAS signal (convoluted with the total volatile hydrocarbon content) and the wet-chemically determined total PAH content of various diesel engines at different loads and speeds.

1.4. SAMPLING WITH SUCTION, WITH DEPOSITION

1.4.1. Discontinuous sampling

A typical technique for the sampling of unstable species is cryo sampling [109], where the species is frozen or condensed within a protective matrix. The condensate is then chemically analyzed and the volume of air sampled is computed by measuring the volume of water collected and assuming the air is totally stripped of water. Open questions relate to the collection of particles by thermophoretic and diffusiophoretic forces which act during condensation. Gases are sampled referentially, especially the soluble ones. A certain drawback is the small volume of water sampled. Some applications are reported in connection with balloon-borne investigations of radicals or pesticides. One recent example is the sampling of herbicide traces by condensing atmospheric water and water-soluble gaseous atrazine [110]. Although only a few microliters of aqueous sample are obtained, the use of an ultrasensitive determination technique, such as an enzyme-linked immunosorbent assay (ELISA) for atrazine, allows the measurement of enriched volatilized pesticide at the stagnant boundary layer for the first time. Extreme absolute sensitivity is the prerequisite for such sampling and analysis (in the given example 100 fg of atrazine is the absolute detection limit). As can be seen from Fig. 1.8, an enrichment of condensable atrazine, a wide-spread herbicide, is observed near the inversion layer. One can conclude that contamination of remote and non-contaminated areas is predominantly influenced by such air-freight processes.

An improved aqueous scrubber (see Fig. 1.9) for collection of soluble atmospheric trace gases, such as NH_3 , HCl or SO_2 , consists of a combination of an aerosol generator and water condenser [111]. During sampling, air is drawn through a nebulizing nozzle, thereby aspirating the absorber solution and forming a "trace catcher" aerosol. The resulting mist sprays upwards through a reaction chamber until it impinges on a hydrophobic

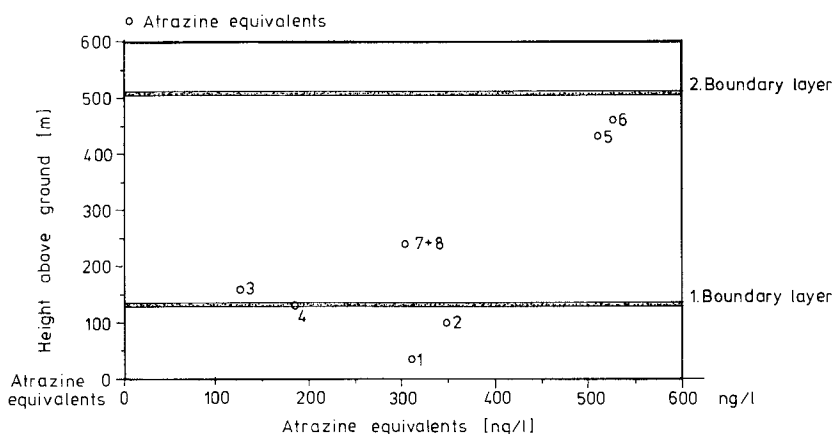


Fig. 1.8. Results of balloon-borne investigation of condensable atrazine traces as a function of height above ground.

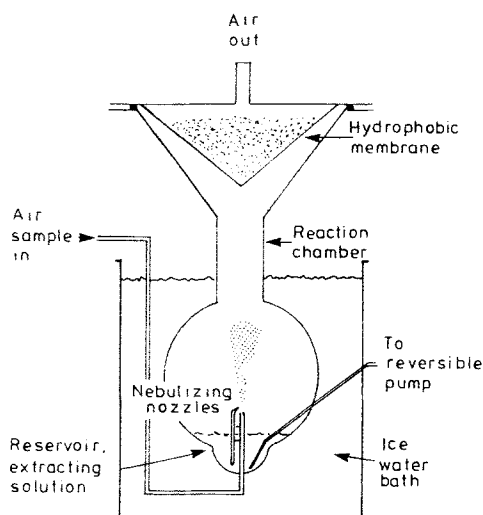
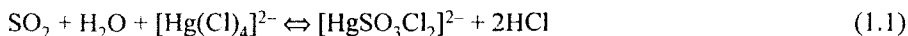


Fig. 1.9. Schematic representation of nebulization-reflux concentrator.

membrane that virtually blocks the passage of droplets but not the gas flow. After a preselected concentration period, the extracting solution containing the analyte can be withdrawn for analysis. A similar application for the determination of chlorine was published by Isacson and Wettermark, who observed the chemiluminescence when a luminol aerosol was reacted with the oxidant [112].

Another example of sampling of highly volatile and unstable aerosol constituents, such as tobacco aerosols or fog droplets has been reported by Keith [113]. He proposed co-condensation or micro-encapsulation of the aerosol under study, using the vapor of methyl cyanoacrylate monomer. During sampling, the monomer starts the polymerization reaction and the whole sampling ends with micro-encapsulated, deposited aerosol particles. This technique seems very advantageous for postcollection inspection by FTIR techniques since the encapsulation is IR-transparent. Similar intentions are seen in the work of Bächmann, in which single rain droplets were allowed to fall through liquid nitrogen vapor and were immediately frozen. Using spot analysis techniques, the single frozen droplets can be chemically characterized [114].

Stabilization of reactive air constituents to avoid artifact formation was attempted by different groups for sulfuric acid aerosols [6,115]. Amine vapors were admitted to the aerosol stream, yielding an amine sulfate that was no longer reactive towards halides. Unfortunately, it was never possible to separate the amine sulfates from other sulfates, such as ammonium salts. Another classical example of a protection step in gas sampling is the well-known West-Gaeke technique, where stabilization is obtained by forming a tetrachloromercurate complex



The use of a proton buffer allows complete preservation of sulfur dioxide against oxidative attack [116].

Gases are usually sampled by chemisorption and/or physisorption. Very often, pre-filters are recommended for removal of particulate material. As discussed in the previous chapter, one has to check carefully that such discrimination does not lead to artifact formation (e.g. by volatilization of already sampled particles, gas/particle interaction or by irreversible adsorption on the filter matrix). Capacity problems have to be considered also, especially in solid sorbent sampling, such as that of organic vapors on charcoal or on materials like Tenax. The subsequent desorption step can also be a source of serious problems due to irreversible adsorption or chemical reactions on the sorbent. Critical testing of the whole sampling procedure (sample transfer through tubing, preconcentration, desorption and determination) with suitable test gases or aerosols is strongly recommended.

Perhaps the most powerful technique for gas/particle separation is diffusion sampling by means of denuder tubes. A known volume of air containing the analyte is drawn through a tube or tube-like arrangement, called a denuder. The inner walls of a denuder are coated with a trapping agent suitable for the gaseous analyte. Gas/particle separation is obtained as the aerosol is drawn through the denuder, since gaseous constituents have a considerably higher diffusion coefficient than even ultrafine particles. This is exemplified in Fig. 1.10, where the relative change of diffusion coefficient as a function of particle size is plotted.

Diffusion tubes have a long tradition. Even in 1900, Townsend estimated the diffusion coefficient of ions in moist gases using denuder tubes [117]. Today a large variety of gaseous and particulate air constituents can be determined using denuder sampling technologies. A comprehensive review on this subject has been published by Slanina et al. [118]. We can categorize these sampling procedures as follows:

- I. Deposition of a gas without any analytical determination
 - use of a wall coating as a sink with limited capacity [119–121]
 - deposition of a gas on a continuously renewed sink [122]
 - catalytic destruction of a gas at the wall coating [123]

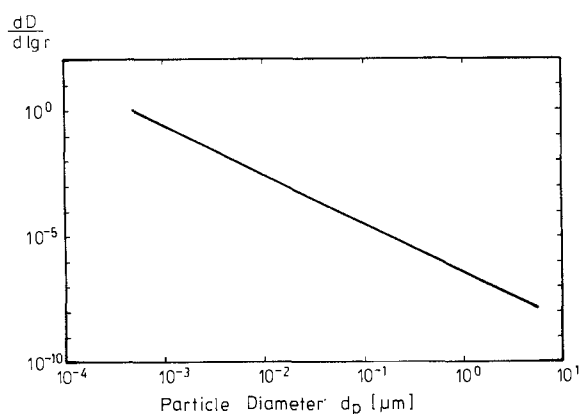


Fig. 1.10. Relative change of diffusion coefficient as a function of particle size.

- II. Deposition of a gas with subsequent analytical determination
 - one-time sampling and analytical determination after elution of the wall coating [124–145]
 - thermodesorption of the adsorbed gas from a reversibly acting sink, with subsequent analytical determination of the gas [146–150]
 - liberation of a new, stoichiometrically formed substance; determination of the deposited gas by analysis of the newly formed compound [105,106,151]
 - determination by parallel sampling with and without a diffusion sampler, (difference determination) [121,152]
 - deposition of a gas on a continuously renewed liquid sink and continuous analytical determination of the adsorbed species [153–157]

Within recent years, a clear trend has been observed in the application of highly selective sink materials. Immobilization of enzymes [158] and the application of antibodies for specific trapping of deposited antigens can be expected in future “super-denuders”.

Diffusion sampling may also help (in favorable cases) in size-related particle/particle sampling:

- I. Diffusion batteries for particle/particle separation
 - in situ determination of particle size distribution (combination with particle counters) [117,159–177]
 - combination of size-fractionating aerosol sampling with deposition and subsequent analysis [178–185]
- II. Thermodiffusion denuder for separation of labile aerosol systems
 - one-time sampling and determination after elution of wall coating [128,130,186]
 - thermodesorption of deposited particulate material from a reversibly usable sink with subsequent chemical analysis [187–191]
 - liberation of a new, stoichiometrically formed compound; determination of the deposited particulate material via analysis of the new compound [128,192,193]
 - determination by parallel sampling, with and without a thermodenuder [194–197]
- III. Separation based on different diffusion coefficients
 - separation of molecules by means of artificially established boundary layers (e.g. membrane or screen) [198–202]
- IV. Separation by change of state (accompanied by drastic change of diffusion coefficient)
 - use of selective gas phase reaction for particle production
 - initiation of particle nucleation by means of electromagnetic radiation
- V. Separation by means of different sinks
 - application of sequentially arranged diffusion tubes of different sink quality [130]

Common to all these diffusion samplers is the principle that proper sampling requires diffusion as the only driving force. In the meantime, many solutions have been published: simple cylindrical tubes, annular denuder, parallel plate denuder, coiled denuder, thermodenuder and solution-based denuder. Of particular note are annular denuders, which have become quite popular. They offer a high operational flow rate and hence a short sampling time for a given minimum amount of enriched material. A drawback compared to the simple straight denuder tube is the large volume required for complete extraction

(because of the extended wall surface of the annular space). This is often a limitation, because of higher blank values. A second serious drawback of all denuder constructions which do not have a straight tube design is associated with an increased particle deposition. Zhang et al. [155] have presented results on particle losses in 8 different denuder configurations, ranging from a simple tube to an annular design. The results clearly indicate a particle loss averaging 3.2–14.7% for a straight denuder tube (particle size range 0.1–2.99 μm ; $Q = 5 \text{ l min}^{-1}$) and of 16.7–19.7% for a glass annular denuder (particle size range 0.6–2.99 μm ; $Q = 5 \text{ l min}^{-1}$).

1.4.2. Continuous sampling

In addition to in-situ sampling (and determination) which has already been mentioned, the quasi-continuously (or even better, continuously) working techniques are of considerable importance, especially for emission control. For many purposes, an on-line measurement (as achieved with optical techniques) is a prerequisite for a self-regulating, and thus optimized, process. A good example is in combustion monitoring where an oxygen sensor is used to establish optimum combustion. For gases, more or less all applicable chemical sensors may be combined. For aerosol monitoring, the continuous transfer of representative ambient aerosol samples, or stack samples containing particles and/or droplets, can give rise to serious technical problems, even at the sampling point, for example using anisokinetic sampling. Anisokinetic sampling leads inevitably to large losses, especially of larger particles with high mass. Since all monitoring of emission sources is governed by mass concentrations (threshold limits), the loss of only a few large particles containing the analyte of interest will yield a false determination.

Simultaneous sampling and analytical determination is used in the total-suspended-dust monitors which work with the β -absorption of the membrane-filter-collected material. Further speciation of the sampled material is not possible here. Another direct total mass recording system uses a piezo-electric quartz sensing element. Deposited material changes the mechanical properties of the quartz crystal, thus changing the resonance frequency in an oscillator circuit.

1.5. CONCLUSION

Sampling is surely the most critical step within the total scheme of air analysis. Because of the arbitrary and complex dynamic nature of air, in terms of the temporal and spatial fluctuations of gaseous and particulate analytes, many sampling problems still exist. Considerable improvements have been achieved within the last decades but with increasing insight into atmospheric aerosol systems, we also see an increased need for faster and more selective sampling techniques which are free of major artifacts. The main conclusion is to avoid sampling (if possible), and to try to exploit the in-situ measurement systems.

A blank space on the map of aerosol sampling and characterization can still be identified easily, i.e. the monitoring of bio-aerosols and microstructured particles.

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Chapter 2

Sample handling strategies for the analysis of organic compounds from environmental water samples

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2.1. INTRODUCTION

With environmental protection high on the agenda of many countries, new rules and regulations are currently being set up for monitoring greater numbers of hazardous organic substances at lower and lower levels. Organic compounds present in environmental waters may be naturally occurring compounds, anthropogenic compounds or degradation products from industrial and urban wastes and agricultural activity. For example, traces of pesticides are regularly detected in surface and ground waters.

Organic compounds are present in environmental water in trace amounts at the microgram per litre (pbb) level or below for most contaminants. They can have very different polarities and chemical properties. In European countries, the drinking water ordinance sets a limit in concentration of $0.5 \mu\text{g l}^{-1}$ for the sum of all pesticides and $0.1 \mu\text{g l}^{-1}$ for each compound, so that detection limits below the $0.1 \mu\text{g l}^{-1}$ level are required for monitoring drinking water. Such low detection limits are also necessary for studying the fate and the transport of organic compounds directly in environmental waters. Screening for low levels of a large variety of compounds requires high performance from analytical instruments as well as sample preparation techniques.

Determination of organic compounds is generally carried out by gas- (GC) or liquid- (LC) chromatography, depending on their polarity, volatility and the risk of decomposition at high temperature. In general, environmental water samples cannot be analysed without some preliminary sample preparation because they are too dilute and too complex. Preconcentration of samples of relatively large volume is necessary to overcome the limitation of the detection system, but the extract is often too complex for an efficient separation by the chromatographic column at low detection levels. Sample pretreatment is therefore an essential part of the whole chromatographic procedure. Its objective is to provide a sample fraction enriched in all the analytes of interest, and as free as possible from other matrix components. This pretreatment, which can be achieved in one to three different steps, consists of (i) extracting traces of analytes of interest from the aqueous

media, (ii) concentrating these traces and (iii) removing from the matrix other components which have been co-extracted and co-concentrated and which may interfere in the chromatographic analysis (i.e. clean-up).

Before implementing any strategy, it is important to consider the strong interdependence of the various steps of the whole analytical procedure: i.e. the sample handling, separation and detection. There is no unique strategy for the sample pretreatment of organic compounds in waters. It mainly depends on the nature of the solutes to be determined (e.g. volatility, polarity, molecular weight), on the nature of the matrix and on the level of concentration required. Interference removal is a critical step which depends strongly on the concentrations of the analytes of interest and on the nature of the aqueous media. In other words, the strategy for determining a pesticide below the microgram per litre level in drinking water will be different from that used for very polluted river water. It will also be guided by the separation, and especially by the method of detection. If a very selective detection can be carried out, the sample handling may be simplified, whereas a simple detection mode can be used if a selective trace enrichment is applied. This "total system" approach is of prime importance for selecting the optimal sample handling strategy [1].

The sample pretreatment is still the weakest link and the time-determining step in the whole analytical procedure, accounting for about two-thirds of the total analysis time. It is also the primary source of errors and discrepancies between laboratories [2]. In the environmental literature, most of the sample preparations described are based on manual and time-consuming procedures that have been used for decades. There is a real need for developing sample-handling strategies which are more rapid, more reliable (which means that the number of intermediate steps such as transfers, evaporation and derivatization is diminished) and easily capable of automation.

Highly water-soluble organic compounds are very difficult to determine at trace levels in water because there is no method for their preconcentration. Slightly to weakly water-soluble organic compounds can be divided into two groups: volatile and non-volatile. Volatile organic compounds are analysed by gas chromatography techniques and their sample pretreatment is carried out using specific techniques. The sample handling of non-volatile organic compounds is different and can be very selective. However, it sometimes requires sophisticated methods, especially if a detection limit of a few nanograms per litre is required in a complex matrix. Trace-enrichment is usually performed by liquid-liquid or liquid-solid extraction techniques which are commonly used off-line. Pretreatment steps are therefore clearly separated from the chromatographic separation. Solid-phase extraction can be also coupled on-line to the chromatographic separation. Gas chromatography is still the most popular analytical technique for the determination of organic pollutants in water. However, liquid chromatography has gained in popularity in recent years owing to its suitability for the determination of polar or non-polar and/or thermodegradable compounds without a derivatization step, but also owing to its automation potential [3,4]. Automatic devices which couple the sample pretreatment, by solid-phase extraction, and the liquid chromatographic separation on-line have recently been introduced by some companies. This offers a fast, modern and reliable approach for monitoring traces of organics in water using a completely automated method with no sample manipulation between the sample percolation and the analysis.

2.2. SAMPLE HANDLING OF NON-VOLATILE ORGANIC COMPOUNDS

The aims in the determination of organic compounds in environmental water samples can be to give a broad-spectrum analysis, with determination and identification of the largest possible number of known and unknown analytes at one time, or the determination of one or several target compounds. The first approach requires a non-selective preconcentration and is straightforward, but the extract is often complex and has to be fractionated before analysis. In the second approach, the selective preconcentration of target analytes is more challenging, and always more rapid.

The sample preparation technique which is still often preferred by environmental chemists, especially as a first approach, is liquid-liquid extraction (LLE). Most of the official methods use it because of its simplicity. LLE is a fully developed technique, well described in the environmental literature, and is briefly reviewed. Solid-phase extraction (SPE), i.e. the enrichment of trace compounds on suitable sorbents, has been shown to be an interesting alternative to LLE and has now become a reliable and useful tool for sample handling. It has already been validated by official institutions such as the US EPA for the determination of some pesticides in water [5,6]. The scope for automation, for on-line coupling with the chromatographic separation, and the possibility of carrying out very selective preconcentrations are the main reasons for further developments. Chemists working in the biomedical field seem to have fewer difficulties in adopting SPE preconcentration than do environmental chemists. About 50% of SPE cartridges are sold for pharmaceutical and clinical applications, but only 10% are for environmental applications. This is probably because separations of biological samples are mainly performed by LC owing to the thermal lability of many of the compounds in biological analyses. Because SPE is based on the LC principles, it appears to be more straightforward to chemists working in this field. Special attention will therefore be devoted to a comprehensive description of the main principles governing SPE in this section.

2.2.1. Liquid-liquid extraction and concentration procedures (LLE)

2.2.1.1. General considerations

Liquid-liquid extraction is based on the partition of organic compounds between the aqueous sample and an immiscible organic solvent. The efficiency of an extracting solvent depends on the affinity of the compound for this solvent, as measured by the partition coefficient, on the ratio of volumes of each phase and on the number of extraction steps. Solvent selection for the extraction of environmental samples is described in many reviews [7–12] and is related to the nature of the analyte. Non-polar or slightly polar solvents are generally chosen. Hexane and cyclohexane are typical solvents for extracting aliphatic hydrocarbons [13] and other non-polar contaminants such as organochlorine or organophosphorus pesticides [14]. Dichloromethane and chloroform are certainly the most common solvents for extracting non-polar to medium polarity organic contaminants [15]. The large selection of available pure solvents, providing a wide range of solubility and selective properties, is often claimed as an inherent advantage of LLE techniques. In fact, each solvent is seldom totally specific toward a class of compounds and LLE is

mainly used for the wide spectrum of compounds extracted. The so-called lipidic fraction is obtained by extraction with chloroform and contains many organic compounds such as alkanes, aliphatic and aromatic hydrocarbons, alcohols, fatty acids and sterols [16,17]. From 1 l of water sample and three extractions with a total volume of 200 ml of dichloromethane, the average extraction recoveries obtained for about 30 commonly applied medium-polarity pesticides are from 60 to 90% [18–20]. The extraction recoveries depend on the spiking level and are higher when samples are spiked with 200 ng l⁻¹ instead of 50 ng l⁻¹ [19]. The recoveries may also be different when measured with spiked pure water samples or with real samples.

LLE can be performed simply, and batchwise, using separated funnels. The partition coefficient should therefore be large because there is a practical limit to the phase-volume ratio and the number of extractions. When the partition coefficient is small and the sample very dilute, a large volume must be handled and continuous liquid-liquid extractors should be used. The extractions then take several hours. Such extractors have been described in the literature [9,20–23].

The partition coefficient may be increased by adjusting the pH to prevent ionization of acids or bases or by forming ion pairs or hydrophobic complexes (with metal ions for example). The solubility of analytes in the aqueous phase can be reduced by adding salts. Fractionation of samples into acidic, basic and neutral fractions can be obtained by successive extractions at different pH [24,25]. A typical scheme is represented in Fig. 2.1. This type of fractionation was applied for the determination of pentachlorophenol in sewage sludge and contaminated waters. No further clean-up of the acidic fraction was

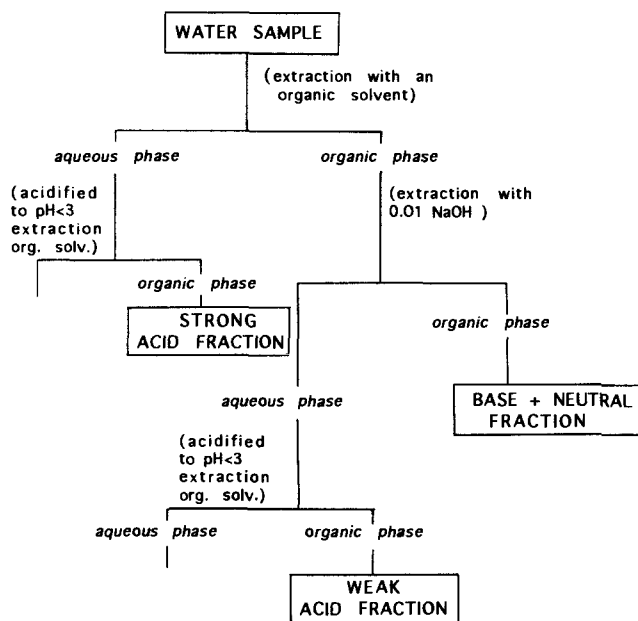


Fig. 2.1. Typical scheme for fractionating water samples into acidic, basic and neutral extracts.

needed and pentachlorophenol was determined by GC using electron-capture detection after simple methylation [26].

It is difficult to compare recoveries obtained by different laboratories because their extraction conditions (pH, phase ratio, number and time-length of extractions, salinity, etc.) are generally different. Sample volumes can be very high, up to 200 l or more [27]. Sample volumes of 50 l of surface water [28] or 20 l of sea water [16] allow the determination of 5 ng l⁻¹ of alkanes. When using a specific detection, the sample volume can be lower: 2 ng l⁻¹ of polyaromatics were determined from 1 l of river water using liquid chromatography and fluorescence detection [29]. Chlorophenols below the 10 ng l⁻¹ level were determined from 100 ml of sea water with electron capture detection coupled to gas chromatography [30].

The LLE of relatively polar and water-soluble organic compounds is, in general, difficult. The recovery obtained from 1 l of water with dichloromethane is 90% for atrazine but lower for its extracted, more polar, degradation products, de-isopropyl- (16%) de-ethyl- (46%) and hydroxy-atrazine (46%). By carrying out LLE with a mixture of dichloromethane and ethyl acetate with 0.2 M ammonium formate, the extraction recoveries were increased to 62, 87 and 65%, respectively, for the three degradation products [31].

2.2.1.2. Concentration procedures

LLE results in the extraction of the sample into a relatively large volume of solvent which can be concentrated using a rotary evaporator, a Kuderna-Danish evaporative concentrator, or some other automated evaporative concentrator, down to a few millilitres. Further concentration down to a few hundred microlitres can be obtained by passing a gentle stream of pure gas over the surface of the extract contained in a small conical vessel. The solvent-evaporation method is slow and has a risk of contamination. Micro-extractors have been described, and have the advantage of avoiding the further concentration of organic solvents [8,12]. One of them allows the handling of an aqueous volume up to 980 ml, extracted with 200 μ l of organic solvent [32]. Although this was applied to the extraction of hydrocarbons, chlorinated pesticides, and phthalate esters, at trace levels, with average recoveries of 90 % after three consecutive extractions, the use of such apparatus is not often described in the environmental literature.

2.2.1.3. Advantages and drawbacks

The main advantages of liquid-liquid extraction are its simplicity and its requirement of simple and inexpensive equipment. However, it is not free from practical problems such as the formation of emulsions which are sometimes difficult to break up. The evaporation of large solvent volumes, and the disposal of toxic and often flammable solvents, are inherent to the method. The LLE requires several sample-handling steps and contamination and loss have to be avoided at every step. There is a risk of exposure of the chemists to toxic solvents or vapours. The glassware equipment must be carefully washed and stored under rigorous conditions. The organic solvents must be very pure and expensive pesticide-grade solvents should always be used when determining traces of pesticides in water.

Carrying out LLE in the field is not easy and large water samples are usually transported and stored in laboratories. Automation of the whole procedure of extraction and concentration requires the use of expensive robots, so it is typically an off-line procedure. Loss during the transfer and evaporation steps always occurs, although to a small extent. Standards are therefore often added before LLE and then the recoveries are calculated from standard peaks by supposing that the losses are similar for solutes and standards. Solubilization of the standards in the samples should be assessed carefully. Losses due to adsorption on vessels are frequently encountered, especially for apolar solutes.

All these factors explain why liquid-liquid extractions are often described as tedious, time-consuming and costly.

2.2.2. Solid-phase extraction-concentration procedures (SPE)

Solid-phase extraction (or liquid-solid extraction) is a sample preparation method which is especially well adapted to the handling of water samples. Trace organics are trapped by a suitable sorbent packed in a so-called extraction column through which the water passes, and are later recovered by elution with a small volume of organic solvent. Extraction and concentration are therefore performed at the same time. This technique appears less straightforward than LLE, because there is a large choice of sorbents and because the recoveries depend on the sample volume. In fact, SPE is simple when one considers that it is based on the well-established separation principles of liquid chromatography.

2.2.2.1. Description

SPE can be used off-line, the sample preparation being completely separated from the subsequent chromatographic analysis, or on-line by direct connection to the chromatographic system.

2.2.2.1.1. Off-line methods

In off-line methodologies, the samples are percolated through a sorbent, packed in disposable columns or cartridges, or enmeshed in an inert matrix of a membrane-based extraction disk. Disposable prepacked columns or cartridges are available from many manufacturers under various trade names such as Sulpeclean, Quick-Sep, Bond-Elut, Baker-Bond, Sep-Pack, Extra-Sep, Hyper-Sep or Extra-Clean. The containers and reservoirs are generally made of polypropylene. The sorbent bed varies from 100 to 1000 mg and is retained between two porous frits. The volumes above the packing vary from 1 to 20 ml in columns designed with large capacity reservoirs. For larger volume samples, the reservoirs can be attached to the columns via an adapter, or directly to the cartridges. Single samples can be processed by attaching a syringe to the SPE column or reservoir for application, and elution. The sample may also be aspirated through the column by vacuum. The size of the bed-packing is between 30 and 75 μm so that a high flow rate can be applied. Another method of application is to use centrifugation by inserting the SPE cartridges into an appropriate centrifuge tube.

Various vacuum manifolds allow batches of up to 24 samples to be prepared simultaneously. The application of samples and solvents in a SPE process can thus be performed semi-automatically, with no risk of sample contamination. Some reservoirs are compatible with the Zymark laboratory robot and the sequence can be totally automated. A typical SPE sequence involves the steps shown in Fig. 2.2. First, the SPE column is prepared to receive a sample, by activation or wetting with a suitable solvent, and by conditioning with water (Fig. 2.2a). Then, the aqueous sample is applied and often the analytes of interest are trapped together with other components (interferences) of the sample matrix (Fig. 2.2b). Some of these interferences can be removed by application of a washing solvent (Fig. 2.2c). This clean-up is examined in more detail later. In the last step, elution of the concentrated analytes is performed by the application of a small volume of organic solvent, which can then be gently evaporated to increase the enrichment factor (Fig. 2.2d).

The use of an extraction disk is particularly easy [33,34]. The membrane is placed in a filtration apparatus attached to a water aspirator vacuum source and the water sample is filtered through the disk after it has been conditioned with 10 ml of methanol and 10 ml of organic-free water. Then the extraction funnel and frit assembly is transferred to a second vacuum filtration flask containing a test-tube. A 5-ml portion of the eluting organic solvent is then drawn through the membrane, with the vacuum being interrupted to allow it to soak the disk for several minutes. This is generally repeated with another 5-ml aliquot of solvent.

Compared with LLE-based sample preparation, the off-line SPE offers reduced processing times and substantial solvent savings. Percolation of samples can be performed in the field and good storage of the adsorbed analytes is generally observed [35]. The problem of transport and storage of voluminous samples is avoided, which is particularly useful when samples have to be taken from remote sites. Automation is possible, using robotic or special sample preparation units that sequentially extract the samples and clean them up for automatic injections. Nevertheless, a certain amount of tedious labour remains and off-line procedures have the inherent disadvantages of loss in sensitivity owing to the injection of an aliquot, of losses in the evaporation step and some risks of contamination, so that internal standards are required.

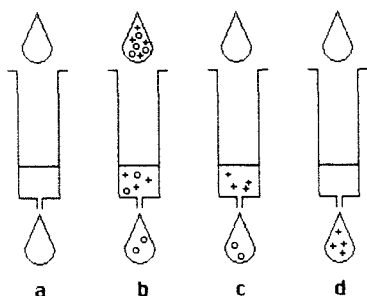


Fig. 2.2. Example of a solid-phase extraction sequence on a disposable column packed with C_{18} silica: (a) conditioning (activation with 3–5 ml of methanol and rinsing with 3–5 ml of deionized water); (b) sample application (+, analytes and O, interferences); (c) clean-up (washing with a mixture of water and organic mixture); (d) elution in a collection tube with pure organic solvent.

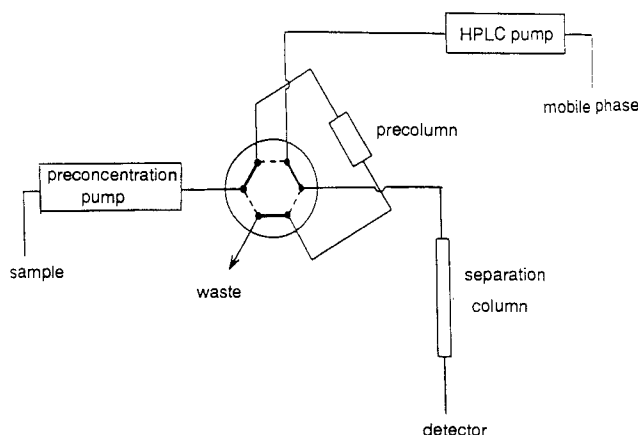


Fig. 2.3. Typical scheme for on-line SPE procedure. The high pressure switching valve is in position 1 (—) for the preconcentration step and in position 2 (-----) for the on-line elution and separation step. From ref. 37.

2.2.2.1.2. On-line methods

On-line coupling of the SPE sample preparation to a GC or LC separation avoids many of the problems mentioned above. On-line approaches coupling SPE to LC are particularly easily performed in any laboratory and are known as column switching or precolumn technology, or on-line multidimensional chromatography. They were extensively developed by Frei and his co-workers [35,36]. A typical scheme [37] for an on-line procedure coupled to liquid chromatography is shown in Fig. 2.3. The extraction precolumn is placed in the sample-loop position of a six-port liquid switching valve. After conditioning, sample application and eventual cleaning via a low-cost pump, the precolumn is coupled to an analytical column by switching the valve to the inject position. The absorbed compounds are then eluted directly from the precolumn onto the analytical column by a suitable mobile phase which also enables the chromatographic separation of trapped compounds. One can expect more accurate quantitative results as there is no sample manipulation between the preconcentration and analysis. Automation is easy and several devices are now commercialized. In contrast with off-line SPE, the entire sample is transferred and analysed, which allows the handling of smaller sample volumes. A more detailed description of the on-line technique is given in the last part of this chapter.

2.2.2.2. Basic principles

The chemistry and principles are essentially identical for both off-line and on-line SPE. To a first approximation, SPE can be considered as a simple chromatographic process, the sorbent being the stationary phase. The mobile phase is the water of the aqueous sample during the extraction step, or the organic solvent during the desorption step. Retention of organic compounds occurs to the extent that they are not eluted by water during the extraction step. Reversed-phase materials are widely used because, in reversed

phase chromatography, water is the less eluting mobile phase for neutral organic compounds. The main sorbents that can be used for retaining organic compounds in aqueous media are reported in Table 2.1. This gives the corresponding separation mechanisms that are involved, the nature of the elution solvent, the characteristics of the analytes concentrated and some applications [37]. The highest enrichment factors are obtained when there is a high retention of analyte by water and a low retention by the desorbing organic solvent. With pure organic solvents, desorption occurs for a volume close to the void volume of the column. From a practical point of view, to obtain high enrichment factors one

TABLE 2.1

DIFFERENT SORBENTS USED FOR SOLID-PHASE EXTRACTION AND ALLOWING PRECONCENTRATION OF ANALYTE FROM A SUFFICIENT WATER SAMPLE VOLUME FOR TRACE LEVEL DETERMINATION; THE CHROMATOGRAPHIC SEPARATION MECHANISM INVOLVED, CHARACTERISTICS OF ANALYTES AND SOME ENVIRONMENTAL APPLICATIONS (FROM REF. 37)

Sorbent	Separation mechanism	Elution solvent	Nature of analyte	Environmental applications
Octadecyl-octyl-bonded silicas	Reversed-phase	Organic solvent	Non-polar and weakly polar	AHs, PAHs, PNAs, PCBs, organophosphorus and organochlorine pesticides, alkylbenzenes, polychlorophenols, phthalate esters, polychloroanilines, apolar herbicides, fatty acids, aminoazobenzene, aminoanthraquinone
Porous styrene-divinylbenzene copolymers	Reversed-phase	Organic solvent	Non-polar to medium polarity aromatic	Phenol, chlorophenol, aniline, chloroaniline, moderately polar herbicides (phenoxyacids, triazines, phenylureas)
Graphitized carbon	Reversed-phase	Organic solvent	Non-polar to relatively polar	Alcohols, nitrophenols, relatively polar herbicides
Silica- and polymer-based ion-exchangers	Ion- exchange	Water (pH adjusted)	Cationic and anionic organics	Phenol, nitrilotriacetic acid, phenoxyacids phenylene-diamines, aniline and polar derivatives, sulphonic acids, phthalic acids, aminophenols
Metal-loaded sorbents	Ligand- exchange	Complexing aqueous solution	Metal complexation property	Aniline derivatives, amino acids, 2-mercaptobenzimidazole, carboxylic acids, buturon

should select the sorbent that gives the highest retention of analytes in water. Breakthrough of solutes occurs when they are no longer retained by the sorbent. Overloading beyond the capacity of the sorbent can also be responsible for breakthrough of analytes [35]. In practical environmental analyses of organic pollutants, where concentrations are typically of the order of micrograms per litre, it is rather unlikely that breakthrough will occur by overloading of the sorbent capacity.

2.2.2.2.1. Breakthrough volume

Figure 2.4 represents a breakthrough curve obtained by monitoring the UV signal of the effluent from an extraction column. A solution of water spiked with an organic compound at trace level and having a UV absorbance A_0 is percolated through a SPE column. Whilst the compound is retained by the sorbent, it is absent from the effluent which will have a UV absorbance of zero. For a volume V_b , usually defined as 1% of the initial absorbance A_0 [38], a frontal or breakthrough curve is recorded, and after a volume V_m , usually defined as 99% of the initial absorbance, the eluate has the same composition as the spiked water solution. Under ideal conditions, the curve has a bi-logarithmic shape and the inflection point is the retention volume of the solute eluted by pure water, V_r , if the column is not overloaded. The quantity V_b corresponds to the sample volume that can be percolated with no breakthrough of analyte.

In trace analysis, the amount of extracted analyte available for detection has to be maximized: it is obtained for a sample volume of V_m (hatched area in Fig. 2.4). Percolation of a higher volume than V_m does not increase the amount extracted. The breakthrough volume, which can be estimated to a first approximation from the retention volume in water [35,39,40], is the most critical parameter for preconcentration. Knowing the concentration limit required ($0.05 \mu\text{g l}^{-1}$ for example) and the absolute detection limit of the chromatographic detection (25 ng injected for example), one can easily calculate the minimum sample volume necessary (500 ml in the example), and therefore obtain a idea of the minimum retention volume, V_r , required.

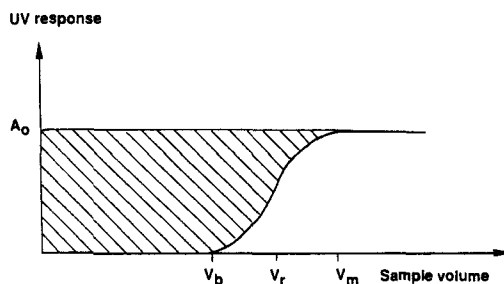


Fig. 2.4. Breakthrough curve obtained by percolation of a spiked water sample with a UV absorbance A_0 through a SPE precolumn; breakthrough occurs for a sample volume, V_b , usually defined at 1% of the initial absorbance; V_r is the retention volume of the analyte and V_m is defined at 99% of the initial absorbance. The hatched area shows the maximal amount that can be preconcentrated. From ref. 37.

2.2.2.2.2. Recoveries

Recovery is defined as the ratio between the amount extracted and the amount percolated. As can be seen in Fig. 2.4, a theoretical 100% recovery can be obtained only for a sample volume equal to, or lower than, V_b . The maximum amount does not correspond to a 100% recovery and is reached for a sample volume equal to V_m . Therefore, the recovery in SPE depends both on the sample volume percolated and on the V_b value, which is related to the chromatographic retention volume in water, V_r , and then to the nature and the amount of sorbent. This explains why recovery values can be compared only if the sample volumes and amounts of sorbent are known. In SPE, it is always possible to show examples with recoveries of 100% by decreasing the sample volume below the corresponding V_b . A simple calculation indicates whether the handling of this volume should allow the required detection or not. Many comparisons between LLE and SPE have been made without taking this parameter into account and sometimes samples up 500 or 1000 l have been percolated.

If recoveries are too low for detection, the only remedy is to increase V_b (or V_r), which can be obtained by increasing the amount of sorbent or choosing another sorbent which will give a higher retention in water for the analytes of interest.

2.2.2.2.3. Determination and prediction of breakthrough volumes

As seen above, the breakthrough volume is the key parameter of the solid-phase extraction, since it indicates the sample volume that can be handled efficiently for the trace enrichment. Although V_b can be measured directly on the recording breakthrough curves, this method is too time-consuming, and can be only performed with an on-line system.

Werkhoven-Goewie et al. [39] demonstrated that V_b can be calculated for any solute from the recording of one frontal curve and the knowledge of k'_w , the capacity of the solute eluted by water and defined by the ratio $(V_r - V_o)/V_o$, where V_o is the void volume of the precolumn.

A faster method for estimating both breakthrough volumes and recoveries has been developed [38,41]. It is easily performed with the on-line set-up. A small volume, V_p , spiked with a known concentration, C_p , of each analyte of interest is percolated through the precolumn. The chromatogram corresponding to the on-line elution of the precolumn is then recorded and the peak areas are measured. This first volume is chosen so that breakthrough does not occur for any solute (5 or 10 ml for instance) and this can be easily verified by comparing the chromatogram with that obtained by loop-injection of the same amount directly onto the analytical column. Then the sample volume is increased and the concentration decreased in order to have the same amount, $C_p V_p$, of analytes in each sample volume, V_p , percolated. Provided breakthrough does not occur for any solute, the amount concentrated remains constant and the peak areas obtained on the on-line chromatogram are constant. When breakthrough occurs, the amount extracted decreases and the peak areas also. Breakthrough volumes can thus be estimated using three or four percolations simultaneously for all the solutes of interest. The corresponding recoveries can also be calculated by dividing the peak areas obtained when percolating a volume V_p by those obtained for 10 ml. By noting A_i , the peak area of one analyte in the first chromatogram without breakthrough, and A_p the peak area of the same analyte when percolating V_p , there is a relation between A_i , A_p and V_b as follows:

when $V_p < V_b$: $A_p/A_i = 1$

when $V_p > V_m$: $A_p/A_i = (V_r/V_p)$

Between V_b and V_m , the relation is not simple, but it has been shown that the relationship above allows a good estimation of V_m and V_r values, as defined in the breakthrough curve [38]. An advantage of this method is that the determinations are performed via the whole on-line system, with the same operating conditions as used for the quantitative analyses.

Figure 2.5a shows experimental breakthrough curves obtained for three herbicides with a 10 mm \times 2.1 mm i.d. precolumn packed with C_{18} silica. These curves are different and spread over a larger volume when the compounds are more retained, owing to the low plate-number in the precolumn. The front corresponding to linuron spreads over nearly 100 ml from a V_b value of 70 ml to a V_m value of 165 ml. First, the determination of V_b at 1% of the initial absorbance on the front of the curves cannot be accurate when the front is not sharp. The second point is that if a 100% recovery is required, the percolated volume has to be lower than 70 ml. Nevertheless, raising the percolated volume to 165 ml considerably increases the amount preconcentrated by nearly 50%. The corresponding recovery is then below 100%, but overcoming the breakthrough volume is sometimes interesting when traces of organic compounds have to be determined in water samples having relatively low organic contamination. Of course, this situation occurs for some analytes when many solutes of different polarity have to be determined together. The volume V_r is then a good indication of the sample volume that can be preconcentrated with good

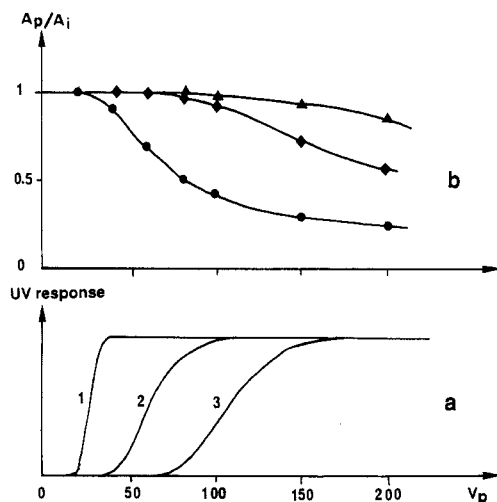


Fig. 2.5. (a) Experimental breakthrough curves recorded with a 1 cm \times 0.21 cm i.d. precolumn packed with RP-18 silica. Samples : solution spiked with 100 $\mu\text{g l}^{-1}$ of simazine (1), atrazine, (2) and linuron (3). (b) Experimental variations of the ratio between peak area (A_p) obtained for the on-line preconcentration and elution of a sample volume V_p spiked at a concentration C_p and peak area (A_i) obtained for a 10-ml percolation volume spiked with 50 $\mu\text{g l}^{-1}$ of each of the three herbicides. The product $C_p \times V_p$ is constant and equal to 0.5 μg . Solutes: \bullet , simazine; \blacklozenge , atrazine; \blacktriangle , linuron. From ref. 38.

recoveries. It is important to note that with on-line methodologies, once the experimental conditions are selected, the quantitative analyses are carried out with spiked samples which are preconcentrated, and are analysed on-line using the whole system. Knowing accurately the recoveries of analytes is therefore useless.

The experimental method derived from the percolation of spiked samples with increasing volumes and decreasing concentration is represented in Fig. 2.5b for the three herbicides together. For simazine, similar values of V_b are found whereas for atrazine and linuron, higher V_b values are estimated from the curves representing the variations of A_p/A_i with the same percolated volume than those measured from the breakthrough curves recorded for each compound separately. This can be explained by the fact that V_b values can be slightly different when analytes are percolated together on the precolumn. A practical advantage is that recoveries and V_b values are estimated for all the analytes of interest with three or four percolations. Similar experiments can be performed using disposable cartridges to estimate V_b values rapidly.

In practice, one first needs an approximate value of V_b for selecting a convenient sorbent and the amount of sorbent, for example in a SPE cartridge. In this way, the curves above indicate that V_r is a good indication of the sample volume that can be handled. This is important because in LC with C_{18} silicas, methods have been extensively studied for predicting the capacity factor in water, k'_w . The value of k'_w can be estimated from the chromatographic measurements of capacity factors using a C_{18} analytical column and elution with mobile phases of mixtures of water and methanol. The advantage is that shorter retention volumes are then obtained. Over a limited range of methanol content, between 15% and 90%, there is a linear relationship between the logarithm of the capacity factor and the percentage of methanol. From rapid measurements with three mobile phases containing different methanol contents, $\log k'_w$ can be estimated by a graphical extrapolation to zero methanol content, with average errors of $\pm 20\%$. The extrapolated values can be lower, similar to or higher than the experimental ones depending on the solutes [42]. Jandera et al. [43] have shown that, for a short microcolumn (30 mm \times 1 mm i.d.) and some solutes, it was possible to measure k'_w directly by elution methods, and that for the more apolar herbicides, the extrapolations are more rapid and more accurate because the experimental range for extrapolation is water-rich. They also proposed a rapid sorption method; here, k'_w is defined as the ratio of the amount of solute in the stationary phase and in the mobile phase. A sample is pumped through a microcolumn until equilibrium is reached, then the amount of solute adsorbed on the stationary phase is measured by desorption onto an analytical column via a switching valve, similar to the on-line preconcentration device. The authors have found that the values determined by the sorption method are higher than those determined by elution methods.

Another method for predicting k'_w is based on the use of the water/octanol partition coefficient, which characterizes the hydrophobicity of a compound and plays an important role in correlating phenomena of physico-chemical, biological and environmental interest [44]. These partition constants, P_{oct} , can be calculated, taking into account the molecular fragment and the intramolecular effects [45]. Braumann [46] has shown a good relation between $\log k'_w$ values, extrapolated from the linear relationship between $\log k'$ and the methanol content, and $\log P_{oct}$. The advantage of this relationship is that k'_w can be approximately known without any measurement.

Since many values of k'_w have been published, the practical problem is to relate them to V_r , which can easily be done using the relationship $V_r = (1 + k'_w)V_o$. The void volume, V_o , is the product of the geometric volume of the precolumn and the porosity ε of the sorbent. An average value between 0.65 and 0.70 was taken for the porosity value of *n*-alkyl-silicas. Cartridges are generally characterized by an amount of bonded silica. With an average density of 0.6 g ml^{-1} for the C_{18} silica used in cartridges, V_o can be estimated as $0.12 \text{ ml per } 100 \text{ mg}$ of sorbent. This value is in agreement with that indicated by Zief et al. who estimated a void volume equal to $1.0\text{--}1.2 \mu\text{l per mg}$ sorbent [47]. Table 2.2 reports the experimental values measured on breakthrough curves and the calculated values from experimental measurements of k'_w . The agreement is satisfactory if one takes into account the fact that for simazine and atrazine the breakthrough curves were recorded for RP-18 silica and that the k'_w values taken from ref. 43 were measured for a different silica. It is well known that the retention varies slightly with the characteristics of the C_{18} silica, i.e. with the number of octadecyl groups bonded at the surface [48].

Table 2.3 reports calculations of retention volumes for apolar to relatively polar organic compounds. The $\log k'_w$ values have been extrapolated using the relationship between $\log k'$ and the percentage of methanol, from our own results [49] or from values in ref. 46. Calculated V_r volumes have been made for an on-line application which uses a $1 \text{ cm} \times 0.2 \text{ cm}$ i.d. precolumn, such as those used in automatic devices or a disposable cartridge containing 100 mg of C_{18} sorbent. It can be seen that the volume, V_r , depends greatly on the hydrophobicity of the solute. For an apolar compound such as phenanthrene, about 3 l of sample can be percolated without breakthrough, whereas for relatively polar compounds such as phenol, aniline, chloroaniline, or nitrophenol, breakthrough occurs with less than 10 ml . Disposable cartridges can contain up to 1000 mg so the calculated volumes can be 10 times higher. Figure 2.6 shows the relationship between values of $\log k'_w$ extrapolated from chromatographic measurements and $\log P_{\text{oct}}$. One can observe that the relationship is correct, allowing the determination of $\log k'_w$ for any compound whose hydrophobicity constant is known or can be calculated. No measurement is then required.

TABLE 2.2

CHARACTERISTIC VALUES, V_b , V_r AND V_m , MEASURED ON BREAKTHROUGH CURVES AND CALCULATED USING EXPERIMENTAL $\log k'_w$ VALUES; SEE TEXT FOR CALCULATION (VALUES FROM REF. 38, OR *FROM REF. 43)

Solutes	Breakthrough curves			Calculated	
	V_b	V_r	V_m	$\log k'_w$	V_r
2-Nitrophenol	1.5	2.5	7	1.9	1.8
Toluene	2.5	5	9	2.45	7 ± 1
Simazine	19	26	36	3.1*	30 ± 3
Atrazine	37	60	110	3.55*	80 ± 7

TABLE 2.3

OCTANOL/WATER PARTITION COEFFICIENTS ($\log P_{\text{oct}}$), $\log k'_w$ VALUES
 EXTRAPOLATED FROM THE RELATION $\log k'$ VERSUS METHANOL PERCENT, OR
 *FROM REF. 44, AND CALCULATED V_r VOLUME (ml) ON (a) A 1 cm \times 0.2 cm i.d. ON-LINE
 PRECOLUMN PACKED WITH C_{18} SILICA OR (b) ON A CARTRIDGE CONTAINING 100 mg
 OF C_{18} SILICA; SEE TEXT FOR CALCULATION

Compounds	$\log P_{\text{oct}}$	$\log k'_w$	V_r (a)	V_r (b)
Pyrene	4.88	5.0	2200	12000
Phenanthrene	4.53	4.4	578	3150
Naphthalene	3.38	3.3	45	245
Ethylbenzene	3.15	3.4	55	300
Toluene	2.76	2.8	12	67
Benzene	2.14	2.2	3.5	20
Fluorobenzene	2.27	2.3	4.5	24
Chlorobenzene	2.84	2.8	13	70
1,2-Dichlorobenzene	3.38	3.4	54	295
Phenol	1.48	1.6	0.8	4.4
2-Chlorophenol	2.16	2.1*	3	15
2,6-Dichlorophenol	2.84	2.8*	12	70
3,5-Dichlorophenol	3.56	3.5*	68	370
2,4,5-Trichlorophenol	4.10	4.0*	200	1094
2-Methylphenol	1.93	1.8	1.5	8.5
4-Nitrophenol	1.91	1.8	1.5	8.5
Nitrobenzene	1.84	2.0	2.5	13.5
1,3-Dinitrobenzene	1.49	1.6	0.9	5
Aniline	0.91	1.1	0.3	1.6
4-Nitroaniline	1.39	1.5	0.7	4
4-Chloroaniline	1.83	1.8*	1.5	8.5
Benzyl alcohol	1.10	1.4	0.6	3.1
Benzoic acid	1.77	1.9	1.4	10
Benzaldehyde	1.45	1.7*	1.2	6.6
Acetophenone	1.70	1.8	1.4	8

The accuracy of these calculations depends on the difference between the real and extrapolated value of $\log k'_w$. It has been shown [49] that the difference is higher for the more apolar analytes and can be positive or negative by up to a factor of 2. Values are closer for solutes with a hydrophobicity constant, $\log P_{\text{oct}}$, below 2. Nevertheless, even with an average accuracy of $\pm 30\%$, the simple calculations of Table 2.3 give a good indication of the suitability of C_{18} for trace enrichment. For example, the determination of aniline at $0.1 \mu\text{g l}^{-1}$ is impossible using C_{18} silicas as sorbent.

2.2.2.2.4. Sample flow rate

Manufacturers recommend the application of water samples at flow rates of 5 ml min^{-1} or less. For large samples, the extraction time can be reduced by applying higher flow

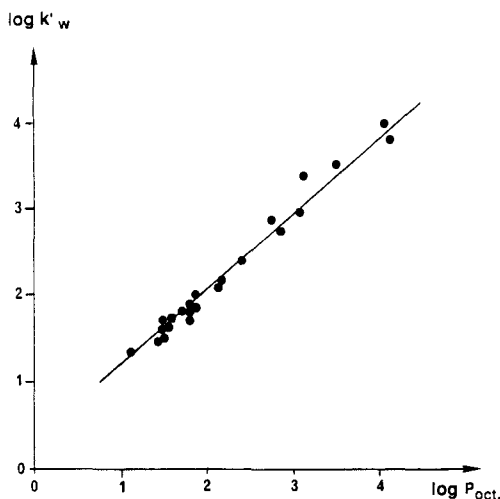


Fig. 2.6. Variations in $\log k'_w$ (values extrapolated from the relationship of $\log k'$ versus methanol content) with the water–octanol partition constants of solutes. Values obtained for RP-18 silica. From ref. 49.

rates because, in theory, breakthrough volumes do not depend on this parameter. For the same reason, it is not necessary to control the flow rate precisely. Some studies have found little correlation between flow rates and recoveries and have shown that flow rates up to 30 ml min^{-1} can be applied with disposable cartridges packed with C_{18} silica or with graphitized carbon black without any loss [50–52]. Flow rates up to 40 or 45 ml min^{-1} have been reported [53] using a cartridge home-packed with a C_{18} silica available for preparative LC and having a $50\text{--}105 \mu\text{m}$ granulometry. Nevertheless, losses in recoveries have been observed with increases in the flow rate. This may be due to channelling effects which may occur, especially at high flow rates, if the cartridge is not packed homogeneously or if it has dried during the conditioning step. It is advisable to apply a low flow rate during the conditioning step and not to allow the sorbent to dry before the sample application.

2.2.2.2.5. Sorbent selection

The choice of the sorbent depends on the volume which must be handled for the required detection and on the nature of the solutes to be determined. Available sorbents that are suitable for extracting organics from water are reported in Table 2.1.

n-Alkyl silicas: These silica-based sorbents, especially C_8 - and C_{18} -bonded silicas, are the most used in analytical and preparative liquid chromatography. They are very pressure-resistant and are available in various granulometry, typically from $3 \mu\text{m}$ to $200 \mu\text{m}$. Their main drawback is their poor stability in very acidic and basic media, which limits their use to the pH range between 2 and 8. Nevertheless, their good reproducibility in retention, rapid equilibrium with mobile phases and rarity of irreversible adsorption of solutes explain their widespread use.

As seen in Table 2.1, a large number of organic compounds, from non-polar to moderately polar ones, are retained by *n*-octadecyl silicas. However, many polar to moderately

polar analytes are not well retained. As already mentioned, retention of analytes can differ from one C_{18} silica to another. It has been shown that the capacity factor of a solute depends on the characteristic of the C_{18} , i.e. on the number of alkyl chains bonded at the surface of the silica [48]. Thus, it is not surprising to obtain different recoveries with C_{18} SPE cartridges having the same amount of sorbent bed but from different manufacturers, or that C_{18} silicas usually provide higher retention than C_8 silicas, and consequently higher recoveries. On the other hand, there has been a real effort from manufacturers to provide more reproducible and cleaner sorbents, so that no contamination can occur from the cartridge.

Many applications using SPE packed with *n*-alkyl silicas have been published. Some of them have been selected owing to the interest given to the proper SPE step

TABLE 2.4

RECOVERIES OBTAINED USING CARTRIDGES AND DISKS; (1) DEPENDENCE OF RECOVERIES (%) OBTAINED BY SPE ON THE SAMPLE VOLUME AND ON THE AMOUNT AND NATURE OF C_{18} CARTRIDGES. (a) BAKER-BOND CARTRIDGES CONTAINING 300 mg OF C_{18} SILICA, SAMPLE VOLUME 100 ml, SPIKE LEVEL FROM 20 TO 500 $\mu\text{g l}^{-1}$ (FROM REF. 18); (b) SUPELCO CARTRIDGES CONTAINING 500 mg OF C_{18} SILICA, SAMPLE VOLUME 2 l, SPIKE LEVEL FROM 0.25 TO 1.5 $\mu\text{g l}^{-1}$ (FROM REF. 63). (2) RECOVERIES OBTAINED USING C_{18} EMPORE™ DISKS FROM 1-l SAMPLES, SPIKE LEVEL 0.5 $\mu\text{g l}^{-1}$ (FROM REF. 34 AND WITH THE PERMISSION OF VARIAN)

Pesticides (1)	Recovery (%) cartridges		Pesticides (2)	Recovery (%) disks
	a	b		
Atrazine	—	96	De-ethylatrazine	55
Carbaryl	90	78	Metoxuron	72
Carbofuran	97	64	Hexazinone	68
Cyanazine	99	95	Simazine	73
Linuron	94	99	Cyanazine	78
Methomyl	25	3.7	Methabenzthiazuron	81
Monuron	98	49	Chlortoluron	76
Oxamyl	28	4	Atrazine	79
Propachlor	90	92	Monolinuron	67
Propham	76	80	Isoproturon	75
Fenitrothion	—	98	Diuron	73
Azinphos ethyl	—	98	Metobromuron	78
Parathion ethyl	—	97	Metazachlor	93
Dinoseb	—	99	Sebutylazine	75
Dinoterb	—	96	Terbutylazine	78
Mecoprop	—	92	Linuron	78
2,4,5-T	—	85	Metolachlor	83
2,4-DB	—	100		

[8–10,18,50–62]. Table 2.4 illustrates the differences that can be observed when the sample volumes and the amount and origin of C₁₈ silicas are different [18,63]. For many pesticides, except polar ones, breakthrough does not occur from a 1-l sample and an average amount of sorbent of 500 mg. Recoveries obtained using filtration of 1-l samples on C₁₈ extraction disks are around 80% for many moderately polar pesticides, except for de-ethylatrazine for which breakthrough has occurred [34].

Another important point is that a drastic reduction in the breakthrough volume can be observed when percolating water samples containing a small content of methanol or another organic solvent. This is a direct consequence of the linear relationship between $\log k'$ and the percentage of methanol. The addition of 1% by volume of methanol to drinking water samples can produce up to a 10% decrease in the breakthrough volume. When spiking the water samples using a standard solution of the solutes in an organic solvent, one has to take care that the final spiked water sample contains no more than 0.1–0.5% of organic solvent.

Porous apolar copolymer-based sorbents: Extraction of organic compounds from water using porous polymers has been reviewed by Dressler [64]. During the last 20 years, Amberlite XAD-type copolymers (mainly XAD-2) have certainly been the most used sorbents for off-line trace enrichment for environmental samples. These styrene-divinylbenzene (ST-DVB) resins are available with different surface areas, granulometry and pore size. Their ability to preconcentrate non-polar to relatively polar analytes has been widely demonstrated [64–77] with various groups of compounds such as polychlorobiphenyl [64,70], aromatic compounds [64], nitro compounds [72], pesticides [69,72,75], organosulphur compounds [76] and humic substances [70,77]. Methacrylate resins (XAD-7 or XAD-8) and Porapak resins were shown to have a higher affinity than ST-DVB resins for polar analytes such as phenol, and methyl and chloro-phenols [64,69,70]. A polyurethane foam which is an amide ester polymer, has been compared with Amberlite XAD-2 for the analysis of hydrocarbons, polychlorobiphenyls and fatty acids dissolved in sea water [74]. The results obtained for both sorbents are similar to those obtained by LLE techniques. The greatest disadvantage of these XAD-type resins is the generation of impurities that are subsequently difficult to eliminate. Resins have to be purified thoroughly before use by extraction in a Soxhlet apparatus. The sequences of flushing-solvents which are commonly recommended are methanol, acetonitrile, then ether [65]. Several purification procedures have been compared and the change of solvent from methanol to water was found to be responsible for impurity release. Desorption with diethyl ether was recommended [70,78].

The fact that these sorbents have to be purified before use explains why prepacked cartridges are not available, except from Supelco who has recently commercialized SPE cartridges containing purified XAD resins. They are not particularly pressure-resistant, and high flow rates should be avoided. For these reasons, these sorbents have not been used as stationary phases in modern liquid chromatography and no chromatographic data are available.

Porous and rigid ST-DVB polymers having similar retention characteristics to XAD-2 [79] have been commercialized for liquid chromatography. These sorbents, PRP-1 (Hamilton) and PLRP-S (Polymer Laboratories) are pressure-resistant, stable over the pH range 1–14, and exist in low granulometry, 7 μm to 15 or 25 μm . Several applications have

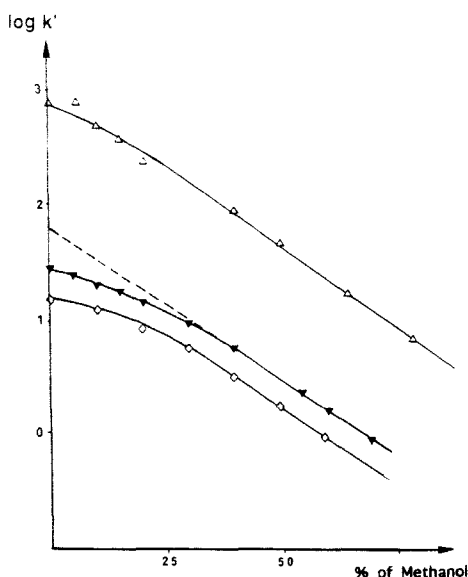


Fig. 2.7. Variation of the capacity factor of benzene with the % of methanol contained in the water/methanol mobile phase. ▼, measured on a 10 cm \times 0.46 cm column home-packed with RP-8 silica; Δ , measured on a 5 cm \times 0.46 cm column home packed with PRP-1 copolymer, \diamond , measured on a 10 cm \times 0.46 cm column of Hypercarb. (From ref. 49)

been reported, using them in precolumns for on-line preconcentration of pesticides and other organic contaminants [35,38,41,80–89].

It would also be interesting to be able to predict V_r values with these sorbents. The retention behaviour of analytes is governed by hydrophobic interactions as with C_{18} silicas, but, owing to the aromatic rings in the network of the polymer matrix, one can expect strong electron-donor interactions (π – π) with aromatic rings of solutes. The behaviour should be also sensitive to changes in the solute electron density caused by the electron donating and withdrawing ability of solute substituents. The retention behaviour of analytes on PRP-1 sorbent has not really been studied and we have tried to compare $\log k'_w$ values obtained with PRP-1 with those obtained with C_{18} silicas. In Fig. 2.7, we report the variations in the capacity factor for benzene with the methanol content of the mobile phase. First, whatever the mobile phase composition, benzene is about 25 to 30 times more strongly retained by PRP-1 than it is by RP-8. The variations of $\log k'_w$ with the mobile phase are parallel, indicating that $\log k'_w$ can be also extrapolated from the relationship between $\log k'_w$ and the methanol content. However, one can expect the relationship between $\log k'_w$ and $\log P_{oct}$ to be less linear, owing to the π – π interactions which are different from one solute to another. Table 2.5 gives the extrapolated $\log k'_w$ values obtained with the PRP-1 sorbent. In comparison to the results obtained with C_{18} silicas, all these solutes are much more retained by PRP-1 than they are by RP-18, and the corresponding V_r values are consequently higher. The difference between the logarithms of the retentions on the two sorbents is reported in the last column of Table 2.5, and varies between 0.9 and 1.66. This indicates that V_r values obtained with PRP-1 are about 25 times

TABLE 2.5

DIFFERENCE ($\Delta \log k'_w$) OF EXTRAPOLATED $\log k'_w$ VALUES OBTAINED FOR C₁₈ SILICA AND FOR PRP-1 SORBENTS (*FROM REF. 44 AND **FROM REF. 41)

Solutes	$\log k'_w$ (C ₁₈)	$\log k'_w$ (PRP-1)	$\Delta \log k'_w$
Ethylbenzene	3.4	4.8	1.4
Toluene	2.8	4.1	1.3
Benzene	2.2	3.5	1.3
Phenol	1.6	2.5	0.9
Benzyl-alcohol	1.4	2.5	1.1
Aniline	1.1	2.5	1.4
Benzoic acid	1.9	3.3	1.4
Benzaldehyde	1.7*	2.9	1.2
Nitrobenzene	2.1	3.6	1.5
Acetophenone	1.8	3.1	1.3
4-Nitroaniline	1.5	2.8	1.3
4-Chloroaniline	1.8*	2.8	1.0
2-Chlorophenol	2.1*	3.2**	1.1
2,6-Dichlorophenol	2.8*	3.9**	1.1
2-Methylphenol	1.8	3.3	1.5
4-Nitrophenol	1.8	2.8	1.0
1,3-Dinitrobenzene	1.6	3.2	1.6

higher than those obtained with C₁₈ silica. The difference is greatest for benzene derivatives substituted by nitro groups which have a strong electron-withdrawing effect, and smallest for the hydroxy group which has a donating effect. The relationship between the extrapolated $\log k'_w$ and $\log P_{oct}$ is, of course, less linear than that with C₁₈ silica [49] and cannot be used to give sufficiently accurate values of $\log k'_w$. Nevertheless, the average factor of (25 ± 12) times higher, verified for many compounds, is important and predictions of the retention volume can, to a first approximation, be derived from those carried out with C₁₈ silicas. Therefore, moderately polar compounds, which are not sufficiently retained by C₁₈ silica can be preconcentrated by these sorbents, PRP-1 or PLRP-S. They can easily be used in on-line methodologies with small precolumns, but are too expensive for use in disposable SPE cartridges. There is a need for manufacturers to provide clean and pressure-resistant porous apolar copolymers, in the range 40–60 μm for off-line preconcentration of more polar organic compounds than those preconcentrated by C₁₈ silicas. Empore™ extraction disks have recently become available, with styrene divinylbenzene copolymer sorbents enmeshed in the matrix (from Analytichem International and J.T. Baker). Table 2.6 gives the percent recovery of EPA Method 625/8270 Analytes from publicly owned treatment works (POTW) water. The sample volume is 500 ml. Except for phenol and 2-fluorophenol, the recoveries are comparable with those obtained with LLE.

Carbon-based sorbents: Activated carbon was one of the first materials used for extracting medium- to low-polarity organic compounds from water. This sorbent was aban-

TABLE 2.6

RECOVERIES (%) OF EPA METHOD 625/8270 ANALYTES FROM PUBLICLY OWNED TREATMENT WORKS WATER (POTW) USING EMPORE™ EXTRACTION DISKS WITH POLY(STYRENEDIVINYLBENZENE) COPOLYMER, 90 mm; FILTRATION OF A 500-ml SAMPLE. COMPARISON WITH RECOVERIES OBTAINED WITH LIQUID-LIQUID EXTRACTION. RSD (%) HAVE BEEN OBTAINED WITH FOUR REPLICATES (FROM REF. 90 AND WITH THE PERMISSION OF J.T. BAKER)

Analytes	Spike level ($\mu\text{g l}^{-1}$)	Empore disk		LLE	
		Recovery	RSD	Recovery	RSD
Nitrobenzene- d_5	200	75	8	76	17
2-Fluorobiphenyl	200	81	9	73	15
Terphenyl- d_{14}	200	94	5	94	8
1,4-Dichlorobenzene	200	63	7	56	17
N-Nitroso-di-propylamine	200	71	3	57	17
1,2,4-Trichlorobenzene	200	70	7	57	17
Acenaphthene	200	81	6	70	18
2,4-Dinitrotoluene	200	85	10	82	18
Pyrene	200	82	3	81	12
Phenol- d_5	400	18	19	77	18
2-Fluorophenol	400	31	11	69	15
2,4,6-Tribromophenol	400	92	22	56	13
Phenol	400	19	8	80	20
2-Chlorophenol	400	71	9	76	17
4-Chloro-3-methylphenol	400	83	11	81	16
4-Nitrophenol	400	76	10	99	12
Pentachlorophenol	400	76	18	68	31

done because irreversible adsorption and low recoveries were obtained for some analytes. Carboxpack B has a non-porous and homogeneous surface with an area of about $100 \text{ m}^2 \text{ g}^{-1}$ and is now available in an SPE cartridge (ENVI-Carb, Supelco). It has been successfully employed for the preconcentration of organic pollutants such as phenols [91], chloroanilines [92], aromatic hydrocarbons [93,94], organochlorine insecticides [95,96] and pesticides [63,97–100]. As with other carbonaceous sorbents, various functional groups are present at the surface of carbons owing to oxygen chemisorption [101]. Taking advantage of the positively charged active centre on its surface, Di Corcia et al. devised a multi-residue method for pesticides in drinking water, based on a complete separation of basic and neutral pesticides from acidic ones [63]. Atrazine and Simazine adsorbed on Carboxpack B have been stored at ambient temperature for 15 days without significant loss in recoveries [99].

Table 2.7 shows the differences found between the recoveries of some rather polar pesticides using a 500 mg cartridge packed with C_{18} silica, and a 250 mg cartridge packed with Carboxpack B, from 2-l samples. Breakthrough occurred for all pesticides on the C_{18} sorbent, but not on graphitic carbon black [63].

TABLE 2.7

COMPARISON OF RECOVERIES (%) OBTAINED WITH (a) CARTRIDGES CONTAINING 500 mg OF C₁₈ SILICA FROM SUPELCO AND (b) CARTRIDGES CONTAINING 250 mg OF GRAPHITIZED CARBON BLACK, CARBOPACK FROM SUPELCO. SAMPLE VOLUME OF 2 l SPIKED WITH 0.25 TO 1.5 µg l⁻¹ OF EACH PESTICIDE (FROM REF. 63)

Solute	C ₁₈	Carbon
Oxamyl	4	89
Methomyl	3,7	98
Chloridazon	18	98
Metoxuron	64	97
Bromacyl	53	96
Monuron	49	100
Carbofuran	64	98
Carbaryl	78	96
Bromoxynil	33	96
2,4-D	41	93

Graphitic carbon black is not sufficiently pressure-resistant to be used in liquid chromatography. Pyrocarbon-modified silicas [102] and pyromodified carbon black [103] were synthesized by Colin et al. as LC stationary phases. Although these materials were not commercialized, their suitability for on-line trace enrichment of chlorophenols [39] and other polar compounds, such as nitrophenol and nitrobenzene [40] has been reported. Recently, a porous graphitic carbon became available as an LC stationary phase [104] under the trade mark Hypercarb. This sorbent shows a reversed-phase behaviour, as can be seen in Fig. 2.7 where the variation of the capacity factor of benzene with the methanol content of the mobile phase is also shown. It is a reversed-phase behaviour in the sense that the retention decreases when the methanol content of the mobile phase increases. The curve corresponding to the porous graphitic carbon (PGC) is also parallel to those obtained with the RP-8 and the PRP-1 sorbents. This indicates that $\log k'_w$ can be extrapolated from the linear relationship $\log k'$ versus methanol content, as for the other reversed-phase sorbents. Owing to its crystalline structure, which is made of large graphitic sheets held together by weak Van der Waals forces, it is often presented as a more retentive reversed-phase sorbent than C₁₈ silica [105]. It has been pointed out that one basic difference between these reversed-phase sorbents is that solute-stationary phase interactions play an important role with carbon-based sorbents whereas they can be neglected in a first approximation with C₁₈ silicas. The affinity of PGC towards very polar and water-soluble polyhydroxybenzenes has been shown [106]. The capacity factor in water of the very polar 1,3,5-trihydroxybenzene (phloroglucinol) is about 1000 with PGC whereas it is 3 with PRP-1. This compound is not retained by C₁₈ silica and was even proposed as an experimental probe for determining the void volume of C₁₈ columns [107]. Other extrapolated or directly determined $\log k'_w$ values have been obtained for mono- and polysubstituted benzene derivatives, and RP-18, PRP-1 and PGC. The results are reported in Table 2.8. First, when we compare values for monosubstituted benzenes, compounds are more

retained by PRP-1 than they are by PGC. The comparison between RP-18 and PGC indicates that solutes are less or more retained by PGC than by RP-18. In contrast to the results on PRP-1, which indicate that retention of all the solutes is higher with PRP-1 than that with C_{18} silicas, no correlation was found between the retention of monosubstituted benzenes on PGC and on C_{18} silicas. The disubstituted benzenes listed in Table 2.8 are rather polar compounds and are either not, or only slightly, retained by C_{18} silicas, which explains why the $\log k'_w$ values have not been reported. The comparisons between the retentions obtained on PRP-1 and on PGC are interesting. With PRP-1, the $\log k'_w$ values obtained for derivatives having two polar substituents are always lower than those measured for each corresponding monosubstituted benzene whereas the contrary is observed with PGC. For example, $\log k'_w$ for 4-aminophenol is 1.1 with PRP-1 and is lower than $\log k'_w$ for phenol (2.4) or for aniline (2.5). With PGC, $\log k'_w$ for the 4-aminophenol is 2.05 and is higher than $\log k'_w$ for both phenol (1.8) and aniline (1.35). The retention mechanisms are therefore different for the two sorbents.

The potential of porous graphitic carbon for trace enrichment of some very polar and water-soluble compounds has been demonstrated by the data in Table 2.8 [49]. Some of these compounds cannot be analysed at trace levels in aqueous samples because they are too water-soluble to be extracted by liquid-liquid extractions, and not retained by other sorbents.

Ion-exchange sorbents: Ionic or ionizable organic compounds can be preconcentrated by ion-exchange sorbents. Only silica-based ion-exchangers are found in disposable SPE cartridges, but have the inherent disadvantage of being limited in the pH range 2–8. They are not widely used for the preconcentration of environmental samples owing to their low

TABLE 2.8

COMPARISON OF EXTRAPOLATED $\log k'_w$ VALUES OBTAINED WITH RP-18 SILICA, PRP-1 AND PGC

Solute	RP-18	PRP-1	PGC
Monosubstituted			
Benzene	2.20	3.50	1.45
Aniline	1.10	2.50	1.35
Phenol	1.55	2.40	1.80
Benzoic acid	1.90	3.20	2.40
Nitrobenzene	2.05	3.60	2.45
Polysubstituted			
4-Aminophenol		1.10	2.05
1,4-Diaminobenzene		1.20	2.40
4-Aminobenzoic acid		2.00	2.85
4-Hydroxybenzoic acid		2.30	2.70
3,5-Dihydroxybenzoic acid		1.35	3.00
1,3-Dihydroxybenzene		1.35	2.35
1,4-Dihydroxybenzene		0.85	2.15
1,3,5-Trihydroxybenzene		0.50	2.70

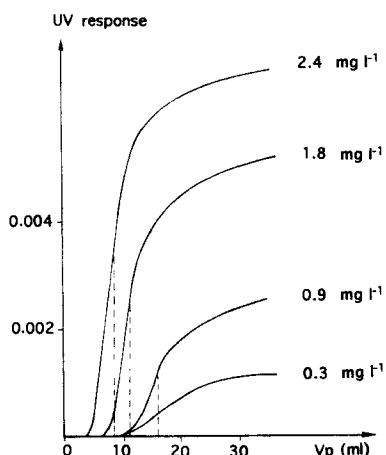


Fig. 2.8. Estimation of the capacity of C_{18} sorbent by recording breakthrough curves from water samples with increasing concentration of dimethyl phthalate on a $1\text{ cm} \times 0.21\text{ cm i.d.}$ precolumn packed with RP-18 silica. From ref. 38.

capacity. Ion-exchange resins are more widely used (Amberlite resins and Dowex resins) and exist in a large range of granulometry. These types are more selective than reversed-phase sorbents. Compounds such as phenols, phthalic acids, carboxylic acids and phenoxy acid herbicides have been extracted by anion-exchangers [108] and anilines, amines or *n*-heterocycles using cation-exchangers. Sorption occurs at a convenient pH, and the analytes are eluted by a change of pH value or of ionic strength with a suitable buffer. For on-line preconcentration, LC-quality ion-exchange resins having low granulometry, $10\text{--}20\text{ }\mu\text{m}$, have been used [80,88,109–111]. The main problem comes from the fact that environmental waters contain high amounts of inorganic ions which overload the capacity of the sorbent. A chemical sample-pretreatment based on precipitation of calcium with oxalic acid and complexation of iron with EDTA has been carried out [112].

Metal-loaded sorbents: Organic compounds which can form complexes with metal ions can be preconcentrated selectively by metal-loaded sorbents. A silica containing the functional group 2-amino-1-cyclopentene-1-dithiocarboxylic acid (ACTA) loaded with platinum(IV) has been shown to irreversibly retain aniline from water [113]. This sorbent was used to remove interfering anilines in the determination of phenylurea herbicides. A mercury/8-hydroxyquinoline phase allowed the preconcentration of 2-mercaptobenzimidazole [114] whereas an Ag(I) -oxine phase was preferred for the determination of buturon in water [115].

Preconcentration on silicas with modification by complexation properties has been reviewed by Veuthey et al. [116]. Some applications of on-line preconcentrations with metal-loaded precolumns have been reported by Nielen et al. [35].

2.2.2.2.6. Capacities of sorbents

One possible cause of breakthrough is the overloading of the capacity of the extraction column or precolumn. Figure 2.8 shows breakthrough curves recorded for increasing concentrations of dimethyl phthalate in water on C_{18} silica [38]. For water spiked with 0.3

and 0.9 mg l^{-1} , breakthrough occurs at the same percolated volumes, V_r , but for higher concentrations the breakthrough volumes decrease and are no longer related to the V_r value. Assuming a Langmuir-type adsorption isotherm, overloading occurs when $20 \mu\text{g}$ of dimethyl phthalate is adsorbed on the precolumn, which corresponds to about 1 mg/g of C_{18} silica. The capacity depends on the size of the solute and on its steric configuration. Under similar conditions, it was estimated for xylene as 4 mg/g of C_{18} silica [38]. Capacity values up to $15\text{--}60 \text{ mg/g}$ of packing material have been reported [35]. Higher amounts have been observed for PRP-1 [117]. Although the total concentration of both solutes and interferences has to be considered, the concentrations of organic pollutants in surface water samples are at the microgram per litre level, so that overloading is unlikely to occur.

2.2.2.2.7. Selection of the desorption solvent

The selection of the eluting solvent depends on the selected sorbent and therefore on the separation mechanism involved, according to Table 2.1. The flow rate during this step has to be low. Higher enrichment factors are obtained with lower elution volumes and the minimum volumes are about two void volumes of the cartridge [47]. Methanol and acetonitrile have been the recommended solvents for the elution of compounds adsorbed on C_8 or C_{18} silicas. The solubility of compounds in the mobile phase plays an important role in reversed-phase chromatography [48] and is a useful guide for selecting the eluting organic solvent. Many hydrophobic compounds have a limited solubility in methanol and acetonitrile and a large volume, $1\text{--}5 \text{ ml}$, is sometimes required for analyte elution. For pesticides, ethyl acetate was found to be more efficient, and many apolar to moderately polar pesticides were eluted in the first $60 \mu\text{l}$ of eluate from cartridges containing 100 mg of C_{18} silicas, with recoveries higher than 90% [51,53].

Dichloromethane, acetonitrile, ethyl acetate, hexane and tetrahydrofuran were investigated for recovering neutral pollutants of the EPA Method 625 from waste water using a C_{18} sorbent [52]. These elution solvents gave acceptable recoveries except for hexane. Ethyl acetate and acetonitrile were preferred because they did not elute the heavy oily aliphatics present in many waste waters whereas dichloromethane or tetrahydrofuran did.

In the SPE sequence, air drying is often applied before analyte elution in order to remove residual water which lowers the volatility of the eluted solvent. This is important when the eluate has to be evaporated because the water is incompatible with the solvent required for further clean-up or for injection into the chromatograph [118]. Marvin et al. [119] optimized this step and described a method for the determination of trace quantities of selected pesticides which resulted in a complete automation of cartridge conditioning, sample loading, cartridge air-drying, analyte elution, eluate blow-down, sample dilution and sample injection steps, with a total analytical time of 30 min and with detection in the range $0.02\text{--}0.9 \mu\text{g l}^{-1}$.

Reversed phase sorbents are often used for preconcentration of ionizable compounds in the molecular form. Desorption from the sorbents can be performed using a solution adjusted to a pH where the analytes are in their ionic form (2 units below or above the pK_A). With ion exchanger sorbents, the analytes are eluted from the SPE column by either adjusting the pH in order to neutralize the charge on the analyte (about 2 units below the pK_A for acidic compounds or above the pK_A for basic compounds) or by using a buffer of

high ionic strength (>0.5 M). The aqueous elution solvent can be modified with a water-miscible organic solvent in order to improve the solubility of some analytes which are less soluble in water in the molecular form than in the ionic form.

2.2.2.2.8. *Effect of the sample matrix*

Matrices of natural and tap waters are very different in chemical composition. Breakthrough volume values are often measured or estimated using LC-quality water and can be modified when salts are added (e.g. in sea water) or when many other organic compounds are present (e.g. in waste water). SPE and LLE have been compared for the determination of neutral pollutants chosen over a wide range of solubility and polarity characteristics. The cartridge extractions of spiked waste water samples yielded recoveries on the same sample using EPA Method 625 and liquid extraction [54]. Recoveries for triazine herbicides and organophosphorus pesticides were found to be equivalent with 1 l of spiked sea water, tap and lake water samples, extracted by SPE with C_{18} sorbents. Application of the procedure to natural water samples gave results that agree well with those obtained by solvent extraction methods [49]. Some loss of atrazine and simazine occurred for 800 ml of surface water spiked at the microgram per litre level and extracted by 50 mg of Carboxen B whereas no loss was observed for the same amount of distilled water or tap water [100]. The authors explained this slight loss by partial saturation of the sorbent with interferences. Breakthrough volumes were measured on a precolumn packed with the PLRP-S apolar copolymer for milli-Q, tap and surface water spiked with some polar pollutants. The V_b values of 2-nitrophenol were 60, 65 and 60 ml, respectively, for the three waters [4]. These results indicate that the SPE methods described for drinking water can be applied to sea water without the likelihood of earlier breakthrough due to the difference in the matrix.

Evidence of possible interference from dissolved organic material was presented [55,74]. Recoveries were found to be lower for water fortified with some pesticides and some humic acids than those obtained with water fortified with pesticides alone. Experiments with ^{14}C -labelled diazinon and parathion suggest the formation of a pesticide-humic acid complex, which is not extracted by the C_{18} sorbent [55]. Nevertheless, the results obtained by SPE and by LLE are very close for an optically adjusted humic acid solution corresponding to 5 ppm of dissolved organic carbon.

Surface waters are generally filtered before percolation to avoid plugging of the column and the partition between dissolved and particulate organic substances is set by the pore size of the filtration membrane. Often, surface water samples are not filtered for the LLE method so that pesticides linked to particulate matter may also be extracted. Using SPE methods, filtration occurs during the percolation, even if not carried out before. For moderately polar pesticides, no difference was found between LLE carried out without filtration and SPE using a C_{18} filtration disk. Table 2.9 indicates the results obtained with a non-spiked surface water from a river polluted with simazine and atrazine [120]. The concentrations of herbicides given by the two methods are very close, indicating that these herbicides are in the free water and not in particulate matter.

2.2.2.3. *Advantages and drawbacks*

In this section, emphasis has been given to the theoretical basis of SPE, in order to be

TABLE 2.9

EFFECT OF THE FILTRATION OF SAMPLES OF SURFACE WATER. CONCENTRATIONS FOUND IN TWO DIFFERENT NON-SPIKED SAMPLES OF SURFACE WATER USING LLE WITHOUT FILTRATION AND SPE TECHNIQUES; LLE, EXTRACTION FROM A 500-ml SURFACE WATER SAMPLE, NON-SPIKED (THREE EXTRACTIONS WITH 50, 25 AND 25 ml OF DICHLOROMETHANE); SPE, FILTRATION OF ANOTHER 500-ml ALIQUOT OF THE SAME SURFACE WATER SAMPLE THROUGH AN EMPORE™ C₁₈ DISK (FROM REF. 120)

Sample	Pesticide	LLE ($\mu\text{g l}^{-1}$)	SPE ($\mu\text{g l}^{-1}$)
No. 1	Simazine	0.29	0.3
No. 1	Atrazine	0.97	1.0
No. 2	Simazine	0.20	0.24
No. 2	Atrazine	0.77	0.92

able to select the key parameters of this method, the sorbent and sample volume. However, SPE is not completely free from practical problems such as the risk of overloading the column by percolating unknown samples with a high content of contaminants, or an early breakthrough due to blocking of the pores. Competitive processes exist between the compounds of interest and the components of the sample matrix. Nevertheless, advantages are more numerous in comparison with LLE :

- simplicity;
- speed, and possibility of predicting the experimental parameters (sample, volume, sorbents);
- sampling in the field, avoiding transport of voluminous samples and allowing easy storage;
- efficiency: no emulsion, purer samples;
- safety: the use and disposal of flammable solvent and the exposure of chemists to toxic solvents are reduced to a large extent;
- low cost: less labour, solvent and transport;
- easy automation and the possibility of on-line coupling with the separation step.

2.2.3. Clean-up

The clean-up is an important step in the determination of organic compounds at low levels and depends, of course, on the complexity of the sample matrix and on the detection method, especially when the analysis is performed with LC. It is less important when carrying out a postcolumn photochemical reaction [121] or a selective detection such as fluorescence [122].

2.2.3.1. Clean-up of total extracts

Extracts obtained by LLE or by SPE on non-selective reverse-phase sorbents contain the target analytes and also many other co-extracted compounds that may interfere in the chromatographic separation. Interferences should be removed before the separation, un-

less a selective detection mode is used. A drastic removal of interferences is not necessary if the concentration of analytes to be determined is higher than that of the interfering compounds. It becomes necessary when determining pesticides in polluted river water below the microgram per litre level. Therefore, an analytical strategy carried out with spiked LC-grade water, at a low level, cannot be applied to river water with the same detection limits due to interferences that can generate a high background in the base line and large peaks at the beginning of the chromatogram [123].

Widely used clean-up methods for extracts are based on fractionation of the extracts by LC. A typical scheme for this procedure is shown in Fig. 2.9. Silica, alumina or Florisil (a synthetic magnesium silicate), packed in cartridges or glass columns, are widely employed for fractionating the extract. Step-elution with solvents of increasing polarity allows a separation into fractions, on the basis of polarity differences. Such a procedure was employed by Valls et al. [124] for the determination of ionic and non-ionic contaminants in urban waste and coastal waters. The fraction F1 was eluted by hexane and contained aromatic hydrocarbons; by adding increasing percentages of methylene chloride, methanol and diethyl ether in the eluting mixture, they could obtain seven fractions containing linear alkylbenzenes and polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs) and waxes, fatty acid methyl esters, alkyl and aryl phosphates and ketones, sterols and, in the last eluted fraction, nonylphenol polyethoxylates. Each fraction is then evaporated and often derivatized prior to GC-mass spectrometry (MS) analysis. This analytical procedure contains so many steps that it is very time-consuming and unsuitable for automation. The only advantage is in broad screening for the identification of unknown compounds. It is not well adapted to the rapid determination of target compounds but has long been the recommended EPA method for the determination of many priority pollutants so is still widely used, with an optimization of the fractionation between interferents and analytes.

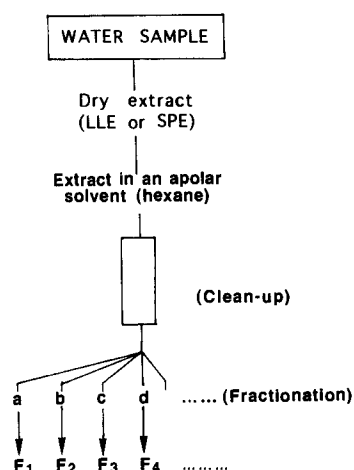


Fig. 2.9. Typical scheme of clean-up for the broad spectrum analysis of water extracts. After injection of the extract, fractionation occurs by eluting the column with eluents a, b, c, d, etc., of increasing polarity.

A more rapid semi-preparative separation of lipid extracts from aquatic media was proposed using liquid chromatography on a silica column [125]. The saponified extract was directly injected onto the column and then eluted by a mobile phase of isooctane containing 0.5–10% of 2-propanol. In a single injection, the following classes could be separated

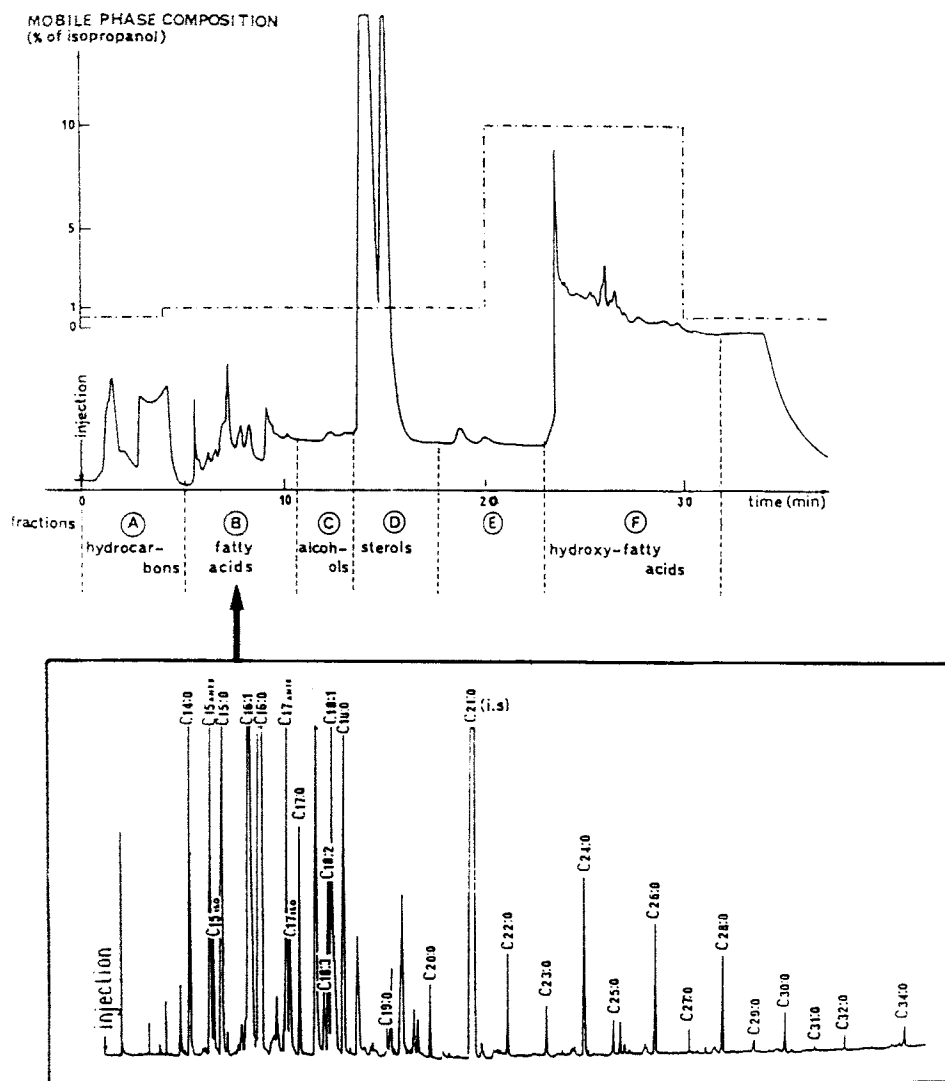


Fig. 2.10. (a) Fractionation of a lipid extract using rapid semi-preparative liquid chromatography of a standard solution after dissolution of dry extract in isooctane with 0.5% of isopropanol and injection of 1.115 ml. (b) Gas chromatogram corresponding to the fraction B obtained with a natural extract after derivatization. From ref. 125.

rated with good resolution: alkanes, aromatic hydrocarbons, fatty acids, alcohols, sterols and hydroxy-fatty acids, as shown in Fig. 2.10.

Clean-up of organochlorine and pyrethroid insecticides has been performed with an automatic unit, the ASPEC (Automatic Sample Preparation with Extraction Columns, from Gilson) [126]. The extract was obtained by LLE from 15 ml of surface water with hexane evaporated down to 1 ml. The clean-up is made with a 100-mg silica cartridge, and the whole sequence (conditioning, sample washing, eluting) is performed by the ASPEC, which has been coupled on-line to capillary GC-ECD by means of a loop-interface equipped with a solvent vapour exit. The complete analytical procedure is greatly facilitated by automation, with a considerable reduction in the sample volume required for the determination of synthetic pyrethroids at the ppt level in surface water.

2.2.3.2. Clean-up included in the SPE sequence

With hydrophobic sorbents, the clean-up step can be included in the SPE sequence as seen in Fig. 2.2. Some of the interferences can be removed by flushing the column with a small volume of water modified with an organic solvent, so that many matrix components are eluted, but not the analytes of interest. This method can easily be applied for non polar analytes but moderately and weakly polar analytes may be eluted with the interferences. It is thus important to verify that no loss occurs with the flushing solvent.

2.2.3.3. Coupling of different sorbents

Di Corcia et al. [92] have shown that when the clean-up cannot be performed after the sample application and before the analyte elution, selective SPE can be performed by coupling two sorbents. The first cartridge, packed with the non-specific graphitized carbon black sorbent traps the analyte of interest and many other compounds, but only basic analytes are transferred and re-concentrated into a second cartridge packed with a more specific sorbent such as a cation exchanger. They could then determine chlorotriazines at the ppt level. By percolating 2 l of drinking water through a cartridge packed with 250 mg of graphitized carbon black and then connecting this cartridge to a second one packed with a cation-exchanger and flushing the two columns with a mixture of dichloromethane and methanol, these authors have also determined 14 phenylurea herbicides in drinking water with detection limits at the nanogram per litre level, as shown in Fig. 2.11. Phenylureas are eluted while all basic interferences such as chlorotriazines and anilines, are trapped by the ion-exchanger [99]. Other examples are shown using on-line techniques.

2.2.4. On-line strategies

The advantages of on-line coupling of SPE to chromatographic separations are mainly in the fact that no risk of loss or contamination exists, and in the potential for automation. The recent commercialization of automatic devices and of sensitive diode array detectors will certainly help further developments in the environmental field. Different aspects of selective on-line coupling with both GC and LC are illustrated in another chapter. In this

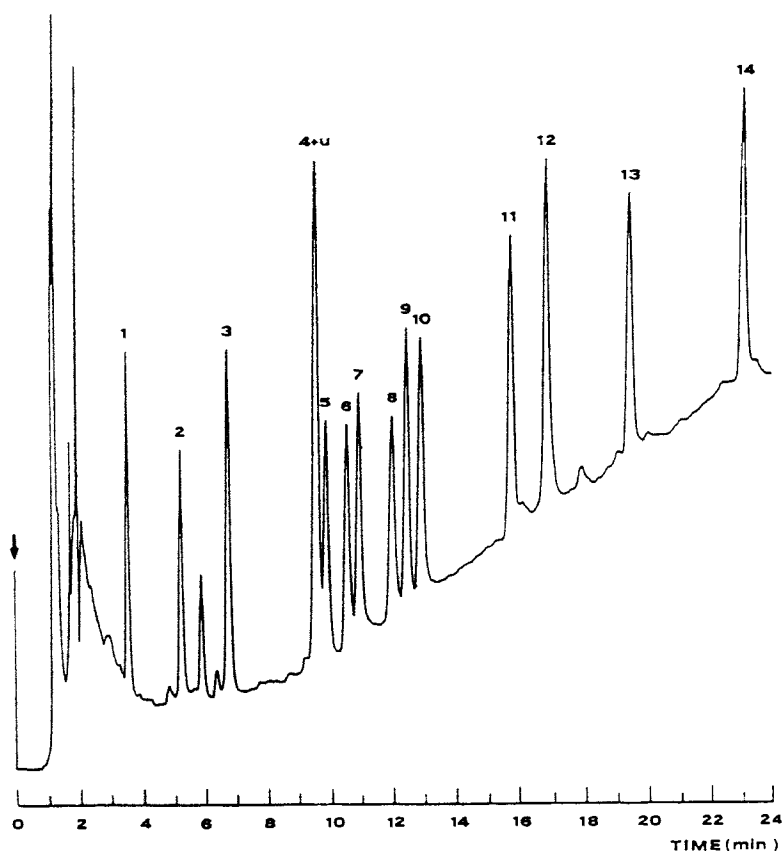


Fig. 2.11. Chromatogram obtained on analysing 2 l of tap water spiked with 30 ng l⁻¹ of each phenylurea. Preconcentration using a SPE cartridge packed with 250 mg of graphitized carbon black and then connection to a second cartridge packed with a cation-exchanger and elution with dichloromethane/methanol. Off-line LC analysis with a C₁₈ silica and a water/methanol/acetonitrile gradient. UV detection at 250 nm. From ref. 99.

chapter, only the characteristics related to on-line SPE (e.g. the size of precolumns, packings and precolumns in series) are presented.

2.2.4.1. Characteristics of the on-line coupling of SPE with LC

A description of the simplest on-line set-up is given in Fig. 2.3. The LC system is often run in the reversed-phase mode, with a C₈ or C₁₈ analytical column and an acetonitrile/water or methanol/water gradient, because the residual water in the precolumn does not have to be removed before desorption. The precolumn is often desorbed by backflushing. By adding a second LC switching valve, there is the possibility both of direct injection onto the analytical column and preconcentration via the precolumn. Comparison of corresponding chromatograms rapidly indicates the quality of the coupling of the

precolumn and analytical column. Loss in efficiency of the analytical column can occur as a result of the coupling and is visible in the larger peaks obtained in the on-line chromatogram compared to those obtained by direct injection into the analytical column. This should not occur if the geometry and packings of the precolumn have been correctly selected.

2.2.4.1.1. Precolumn size

Band-broadening has to be considered with the on-line set-up with a precolumn. That the size of the precolumn is an important parameter is easy to understand because the profile of concentrated species transferred from the precolumn to the analytical column should ideally be as narrow as possible at the beginning of the separation. Consequently, the precolumn should be as small as possible but this depends on the size of the analytical column. Nondek et al. have proposed a relationship giving the optimal volume of a precolumn as a function of the volume and plate number of the analytical column [127,128]. For a classical analytical column of 15 cm \times 0.46 cm i.d., the length of the precolumn should be a maximum of 1 cm, and the diameter smaller than that of the analytical column. Our own experience indicated that taking a very small precolumn was important if the separation is carried out with an isocratic mobile phase. With a methanol or acetonitrile gradient, the dimension of the precolumn can be slightly increased to 1.5 cm and band-broadening can be reduced or removed by applying a convenient gradient. This is why one should be able to control the quality of the coupling by comparing the direct and on-line chromatograms.

A limitation of the small precolumns used in on-line SPE is that it is impossible to increase the breakthrough volumes of analytes by increasing the amount of sorbent. The calculations in Table 2.3 were made for a 1 cm \times 0.2 cm precolumn, as used in automatic devices. By increasing the size of analytical column to 25 cm, the size of the precolumn could be increased and the values in Table 2.3 can therefore be increased by up to a factor of 10. If a higher retention is required, the only solution is to select another sorbent providing higher retention volumes in water for the analytes of interest. This is why, in the on-line methodology, it is particularly important to know the retention behaviour of the analytes with the different sorbents that can be used. One advantage of the small precolumns is that they can be packed with any LC-grade sorbent, even the most expensive ones. In many of the procedures described, they are easily regenerated at the end of each run and therefore can be used several times.

2.2.4.1.2. Sorbents

The sorbents should ideally be the same as in the analytical column, i.e. with a 5- μ m granulometry in most cases. However, in order to have a high sampling rate during the percolation of samples, a higher granulometry of 10–15 μ m has been used without producing band broadening. Empore membrane extraction disks have been placed in a special holder for on-line enrichment with the advantage of low pressure and high stability after repeated use [129].

It is also often said that the analyte retention should ideally be similar to, or lower than, that on the analytical column. That means that the combination of PRP-1 or PLPR-S in precolumns with C₁₈ analytical columns should be avoided. The PRP-1 analytical col-

umns show less efficiency than alkyl-silica columns with water-rich eluents, so that on-line coupling with PRP-1 precolumns is not always efficient. The combination of PRP-1 or PLRP-S with C18 silica columns is unavoidable for the on-line trace enrichment of moderately polar compounds. There again, the band broadening can generally be reduced or removed by the application of a mobile phase gradient, which causes a peak compression effect on the top of the analytical column. This is illustrated in Fig. 2.12, where the precolumn has been increased up to a maximum size of 2.2 cm \times 0.46 cm i.d. and packed with 10- μ m PRP-1 sorbent coupled to a 25 cm \times 0.46 cm i.d. analytical column packed with octyl-silica. The chromatogram represented in Fig. 2.12b corresponds to the pre-concentration and on-line elution of 50 ml of LC-grade water spiked with 5 μ g l⁻¹ of each analyte. When comparing with the chromatogram obtained by direct loop-injection of 20 μ l into the analytical column (Fig. 2.12a), a slight band-broadening is observed for the

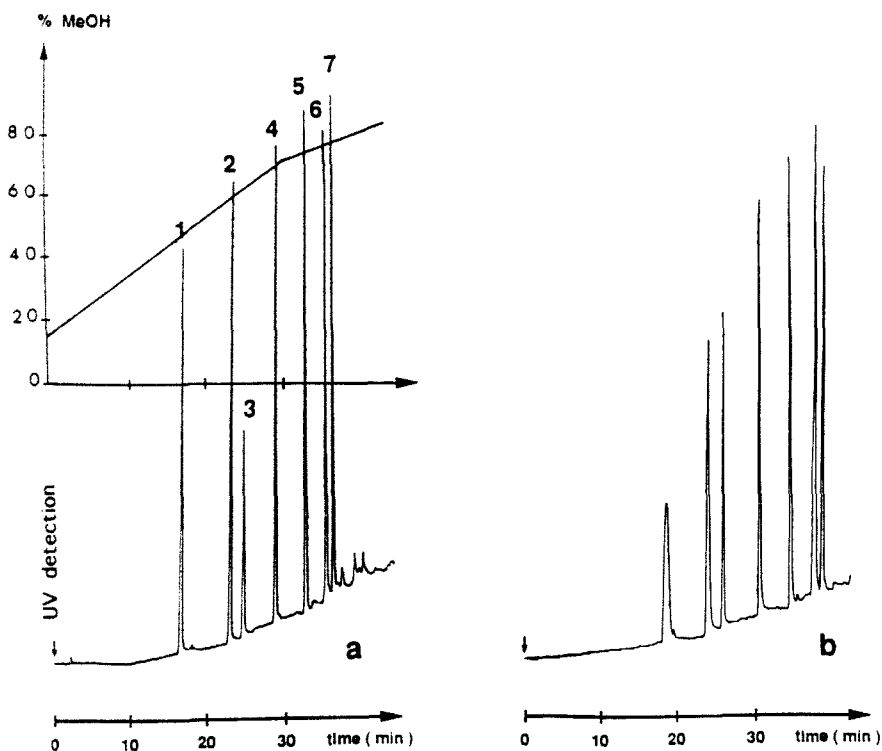


Fig. 2.12. Efficiency of the on-line coupling of a large precolumn packed with PRP-1 with a 25-cm long analytical column packed with C₁₈ silica. Comparison between a direct 20- μ l loop injection (a), and the on-line pre-concentration of 50 ml of LC-grade water spiked with 5 μ g l⁻¹ of each compound (b). Solutes: 1, de-isopropylatrazine; 2, de-ethylatrazine; 3, hydroxy-atrazine; 4, simazine; 5, atrazine; 6, propazine; 7, terbutylazine. Analytical column: 25 cm \times 0.46 cm i.d. pre-packed with 5- μ m C₈ silica, C₈ Zorbax. Precolumn: 2.2 cm \times 0.46 cm i.d. packed with PRP-1 copolymer. Mobile phase: water/methanol gradient with a potassium phosphate buffer at pH 7 as represented. Flow rate: 1 ml/min., UV detection at 220 nm. From ref. 49.

two first (polar) peaks, but not for the other products (Fig. 2.12b) The band-broadening visible with isocratic elutions, or with a step in the gradient [130], was eliminated by the relatively strong increase in methanol shown in the shape of the gradient in Fig. 2.12a.

2.2.4.1.3. *Quantitative analyses*

With an on-line system, it is not advisable to carry out quantitative analyses by comparison with direct injection. First, the volume of many injection-loops is specified to an average accuracy of 20% and calibration of the loop is rather a delicate operation. Secondly, slight, but imperceptible band-broadening may exist and calibration curves made by concentration of spiked samples are more accurate. Once the sample volume is selected calibration graphs should be made with spiked samples, under the same conditions as selected for the analyses of unknown samples. Accurate knowledge of recoveries is then not necessary. However, even if the loop volumes are approximate, the possibility of direct loop-injection of an amount close to that contained in spiked samples is useful in selecting the experimental conditions. With a rapid preconcentration of three increasing volumes containing constant amounts, breakthrough can be estimated for all the analytes at the same time. The efficiency of the spiking can also be assessed. The solubilization of apolar compounds may be only partial and this can also be rapidly detected by comparison with direct injection. If this is the case, it can be observed that the peak areas increase when samples of constant amount but with increasing volume and decreasing concentration are percolated [38].

2.2.4.2. *On-line sample handling with precolumns in series*

Two or three precolumns can be coupled in series for the percolation of the samples, with the advantage of prefractionation or simplification of a complex mixture during the preconcentration step.

2.2.4.2.1. *Fractionation in polarity groups*

A pre-fractionation into polarity groups (non-polar, moderately polar and ionic compounds) was performed using different types of sorbents from series such as C₁₈ silica, PRP-1 copolymer and cation-exchanger sorbents [80]. Table 2.10 shows the breakthrough volumes of 29 selected pollutants on various precolumns. Non-polar analytes (nos. 20–29) were trapped on the C₁₈ silica; medium polarity compounds (nos. 12–19), not retained by the C₁₈ silica, were preconcentrated by the PRP-1 precolumn, and cationic organic compounds by a cation-exchanger precolumn. The water sample was adjusted to pH 3 before percolation so that aniline derivatives (nos. 1–11) were in their ionic form and were not (or only slightly) retained on both RP18 and PRP-1. In order to avoid overloading of the cation-exchanger with the high amount of inorganic ions contained in natural waters (such as calcium or magnesium ions and metallic cations), a chemical pretreatment of the samples was performed. Inorganic cationic interferences were removed by precipitation and complexation by oxalate and EDTA. Using an experimental set-up with four switching valves, each precolumn was separately eluted using a continuous buffer/methanol gradient and separated on one C₁₈ analytical column with diode-array detection. A chromatogram corresponding to the preconcentration of a 5-ml standard solution

containing $200 \mu\text{g l}^{-1}$ of each compound is shown in Fig. 2.13. This fractionation was applied to industrial wastewater analysis. The required detection limits were above $10 \mu\text{g l}^{-1}$ so that the handling of a 10-ml sample was sufficient.

TABLE 2.10

BREAKTHROUGH VOLUMES (ml) OF 29 SELECTED POLLUTANTS ON SHORT PRECOLUMNS PACKED WITH VARIOUS SORBENTS, RP-18 ($10 \mu\text{m}$, PRECOLUMN: $2 \times 4.6 \text{ mm i.d.}$), PRP-1 ($10 \mu\text{m}$, PRECOLUMN: $2 \times 4.6 \text{ mm i.d.}$), AMINEX A5 ($9\text{--}13 \mu\text{m}$, PRECOLUMN: $4 \times 4.6 \text{ mm i.d.}$); LC-WATER SAMPLES CONTAINING 250 ppb OF TEST SOLUTES; pH ADJUSTED TO 3.0 WITH PERCHLORIC ACID; SAMPLING RATE 5 ml min^{-1} ; (–) NOT DETERMINED (FROM REF. 80)

No.	Compound	Breakthrough volume		
		RP-18	PRP-1	Aminex A5
1	<i>p</i> -Aminophenol	0	0	>100
2	<i>p</i> -Phenylenediamine	0	0	>100
3	<i>m</i> -Phenylenediamine	0	1	>100
4	4-Methyl- <i>m</i> -phenylenediamine	0	1	>100
5	<i>o</i> -Phenylenediamine	0	1	>100
6	Aniline	0	2	>100
7	<i>p</i> -Anisidine	0	1	>100
8	<i>p</i> -Nitroaniline	1	10	>100
9	3-Amino-4-ethoxyacetanilide	1	7	>100
10	<i>o</i> -Anisidine	1	6	>100
11	<i>o</i> -Toluidine	1	3	>100
12	Picramic acid	2	>100	–
13	<i>p</i> -Chloroaniline	2	30	–
14	<i>p</i> -Nitrophenol	1	25	–
15	3,5-Dinitro- <i>o</i> -cresol	10	>100	–
16	<i>m</i> -Cresol	1	37	–
17	Nitrobenzene	2	>100	–
18	<i>p</i> -Chlorophenol	2	72	–
19	<i>p</i> -Chloronitrobenzene	3	>100	–
20	Pentachlorophenol	>100	–	–
21	<i>o</i> -Dianidine	10	–	–
22	2-Aminoanthraquinone	>100	–	–
23	3,3'-Dichlorobenzidine	72	–	–
24	3-Amino-9-ethylcarbazole	50	–	–
25	<i>p</i> -Aminoazobenzene	>100	–	–
26	<i>l</i> -Aminoanthraquinone	>100	–	–
27	<i>p</i> -Dichlorobenzene	17	–	–
28	2-Phenylaminonaphthalene	>100	–	–
29	1,2,5-Trichlorobenzene	>100	–	–

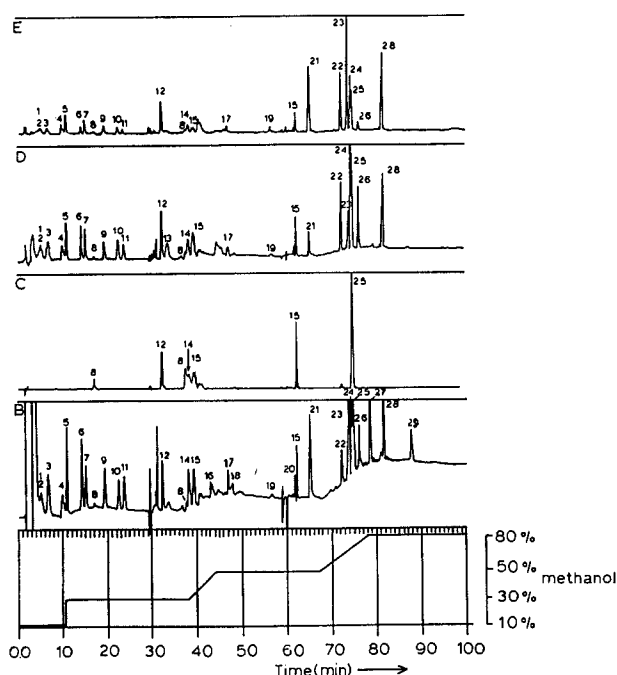


Fig. 2.13. Multi-signal plot of a 5-ml standard solution containing 200 ppb of 29 selected pollutants; gradient elution with 0.1 M potassium acetate (pH 6) and methanol (10–80%) as indicated; preconcentration using three precolumns in series and on-line-eluted separately; the first 28 min correspond to the ion-exchanger precolumn, the period 28–58 min to the PRP-1 precolumn and the final period to the C_{18} precolumn; detection at 220 nm (B), 390 nm (C), 244 nm (D) and 295 nm (E), 0.2 a.u.f.s; peak numbers correspond to the compounds listed in Table 2.10. From ref. 80.

2.2.4.2.2. Interference removal

A sample volume of at least 10–20 ml is necessary for detection limits of $5 \mu\text{g l}^{-1}$ with UV detection. The problem of breakthrough is not critical. Monitoring of drinking water is different because the required detection limits are below $0.1 \mu\text{g l}^{-1}$ and the sample volume must then be at least 100 ml. The concentration of up to 500 ml of tap water was performed on two precolumns in series, the first one packed with C_{18} silica and the second with PRP-1. Breakthrough cannot be avoided on the C_{18} precolumn, as shown in Fig. 2.14 which represents the on-line elution of the C_{18} precolumn and of the PRP-1 precolumn after percolation of a 500-ml sample of LC-grade water spiked with $2 \mu\text{g l}^{-1}$ of the six phenylureas. Breakthrough volumes on C_{18} precolumns have been estimated at about 50 ml, whereas no breakthrough occurs on PRP-1 [87]. About 80–90% of herbicides are indeed trapped on PRP-1. When handling surface waters containing a large amount of organic material, such a fractionation is useful in quantitative determinations below $0.5 \mu\text{g l}^{-1}$. The first C_{18} precolumn acts as an interferent-filter for many apolar compounds and the second traps the analytes of interest. This is clearly shown in Fig. 2.15. Owing to the filtration of many interferences, a much lower background is obtained in the base-line

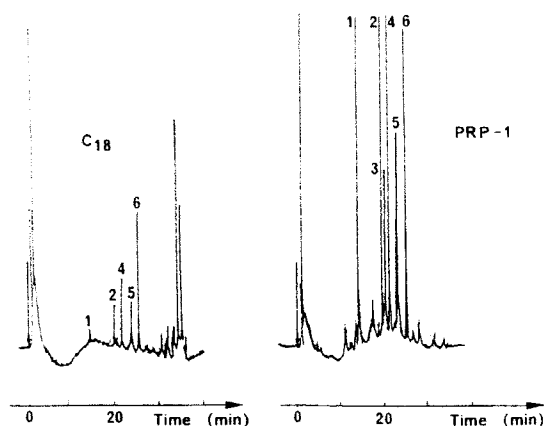


Fig. 2.14. On-line preconcentration of a 500-ml water sample with $2\mu\text{g l}^{-1}$ of each analyte after percolation through two precolumns in series (1 cm \times 0.21 cm i.d.) packed with RP-18 silica and PRP-1 copolymer. On-line elution of both precolumns on a 15 cm \times 0.46 cm i.d. C_{18} column with a water/acetonitrile gradient. Solutes: 1, metoxuron; 2, monolinuron; 3, buturon; 4, chlortoluron; 5, diuron; 6, linuron.

corresponding to the on-line elution of the PRP-1 precolumn compared to that of RP-18. Recoveries are, of course, below 100% since some of the compounds are preconcentrated on the C_{18} precolumn, but the quantitative analyses are carried out by spiking samples and percolation through the two precolumns and then elution of only the PRP-1 precolumn. This method was applied to the monitoring of phenylureas and chlorotriazines in river water. As can be seen in Fig. 2.16, the detection limits obtained are in the low microgram per litre range.

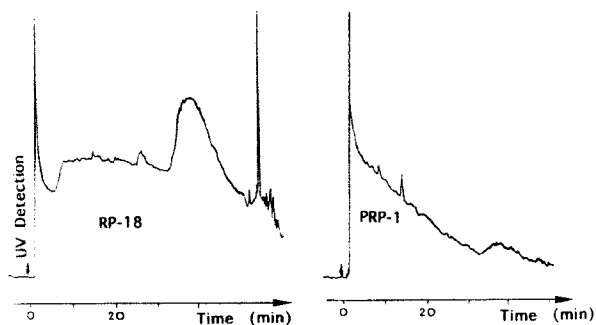


Fig. 2.15. Filter effect of the RP-18 precolumn when percolating 500 ml of a raw river water sample through two precolumns in series packed with RP-18 and PRP-1. On-line elution of both precolumns on a 15 cm \times 0.46 cm i.d. C_{18} column with a water acetonitrile gradient. UV detection at 254 nm, 0.032 a.u.f.s.

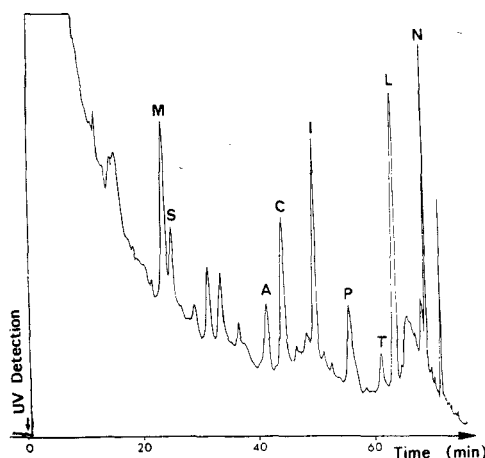


Fig. 2.16. On-line analysis of a 500-ml Rhone river water sample spiked with $1 \mu\text{g l}^{-1}$ of each phenylurea (metoxuron, chlortoluron, isoproturon, linuron, neburon) and triazine (simazine, atrazine, propazine, terbutylazine). Preconcentration through two precolumns in series packed with RP-18 and PRP-1. The chromatogram corresponds to the on-line elution of the PRP-1 precolumn on a C_{18} silica column ($15 \text{ cm} \times 0.46 \text{ cm}$) with a water/acetonitrile gradient; UV detection at 244 nm, 0.04 a.u.f.s.

2.2.4.2.3. Use of ion-exchangers

It was seen above that natural water samples can be percolated through precolumns packed with cation-exchangers provided there is a chemical pretreatment by precipitation with oxalate and complexation with EDTA, to remove inorganic cationic interferences. However, even with this pretreatment, one should not percolate more than 50 ml through a $1 \text{ cm} \times 0.2 \text{ cm}$ precolumn. No chemical treatment exists to remove inorganic anions from water samples. Ion-exchangers are selective sorbents that cannot be used in the direct percolation of large volumes of water. For ionizable analytes in the pH range 1–13, a two-step preconcentration proposed by Nielsen et al. [112] can be performed, which avoids the direct percolation of samples through ion-exchangers. It is based on the fact that solutes are retained on the PRP-1 sorbent when in their neutral form but not in their ionic form. This was applied to chlorotriazines [88] having ionization constants between 1.6 and 2. The water sample adjusted to pH 6–7 was percolated through a single precolumn packed with the PRP-1 copolymer. Breakthrough volumes of the triazines have been estimated to be above 500 ml, from the size of the precolumn ($1.5 \text{ cm} \times 0.32 \text{ cm}$). Then, this precolumn was coupled to a second cation-exchanger precolumn ($1 \text{ cm} \times 0.21 \text{ cm}$) and a small volume (3 ml) of well deionized water with 25% of acetonitrile allowed the triazines to be desorbed from the PRP-1 precolumn and transferred onto the ion-exchanger which was then eluted on-line. The chromatogram in Fig. 2.17 shows the low detection limits, less than 5 ng l^{-1} obtained by this selective sample-handling method. Although no breakthrough occurs for the chlorotriazines on the PRP-1 precolumn, the use of a single PRP-1 precolumn could not provide these low detection limits due to the numerous interferences. Monitoring of drinking water at the $0.1 \mu\text{g l}^{-1}$

level can be performed automatically from a sample volume as low as 50 ml. The first precolumn in these experiments is packed with the PRP-1 copolymer because it is stable over the 1–14 pH range and shows higher retention than C_{18} silica.

This methodology can also be applied to more polar ionizable compounds by increasing the size of the PRP-1 precolumn, which does not need to be eluted on-line. With a $9\text{ cm} \times 0.46\text{ cm i.d.}$ PRP-1 precolumn, compounds such as aniline and some polar derivatives were determined below the $0.1\text{ }\mu\text{g l}^{-1}$ level from a 150-ml drinking water sample [110]. With a $2.2\text{ cm} \times 0.46\text{ cm}$ PRP-1 precolumn, simazine and atrazine have breakthrough volumes higher than 1000 ml whereas their degradation products are much more polar. Breakthrough volumes of de-isopropyl, de-ethyl and hydroxy-atrazine were measured as only 80, 100 and 150 ml, respectively, so that a volume of up to 150 ml can be handled. Using this PRP-1 precolumn and the two-step preconcentration with the cation exchanger, the monitoring of drinking water at the $0.1\text{ }\mu\text{g l}^{-1}$ level was possible [130]. If one wants lower level detection limits in order to study the transport and fate of atrazine directly in environmental waters, or to follow its degradation or transformation in a drinking water treatment plant, the only solution is to again increase the size of the PRP-1 precolumn. By selecting a $5\text{ cm} \times 0.6\text{ cm i.d.}$ precolumn, the detection limits in river water containing a high content of organic material are below 5 ng l^{-1} , as show in Fig. 2.18.

Anion-exchangers can be used similarly; this was applied to the trace determination of phenol [112] and of phenoxyacid herbicides [111]. The water sample is acidified so that the compounds are in their neutral form before percolation through the PRP-1 precolumn, and the desorption and re-preconcentration are performed at about 2 pH units above the ionization-constant value.

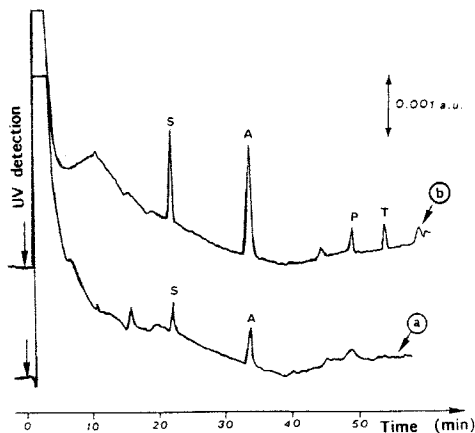


Fig. 2.17. Preconcentration and on-line LC separation of 500 ml of a drinking water sample: (a) non-spiked and (b) spiked with 20 ng l^{-1} of simazine, atrazine, propazine and terbutylazine; preconcentration through a $1.5\text{ cm} \times 0.32\text{ cm i.d.}$ PRP-1 precolumn, transfer to $1\text{ cm} \times 0.21\text{ cm i.d.}$ cation-exchanger precolumn by 3 ml of 0.1 M perchloric acid solution modified with 25% of acetonitrile. On-line elution of the cation-exchanger precolumn to a $15 \times 4.6\text{ cm}$ C_{18} analytical column with a water/acetonitrile-gradient. From ref. 88.

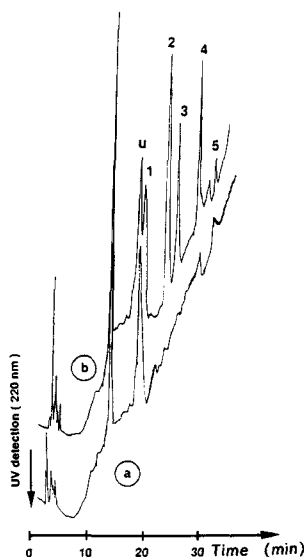


Fig. 2.18. On-line analysis of a 500-ml river Seine water sample: (a) non-spiked and (b) spiked with $0.05 \mu\text{g l}^{-1}$ of each compound. Preconcentration using a $5 \text{ cm} \times 0.6 \text{ cm i.d.}$ PRP-1 precolumn and transfer to the cation-exchanger precolumn ($1 \text{ cm} \times 0.21 \text{ cm i.d.}$) with 4 ml of perchloric acid solution at pH 1 containing 35% of acetonitrile. On-line elution of the cation exchanger to a $25 \text{ cm} \times 0.46 \text{ cm i.d.}$ C_{18} column with a methanol/water gradient; UV detection at 220 nm, 0.08 a.u.f.s. Solutes: 1, de-isopropylatrazine; 2, de-ethylatrazine; 3, hydroxyatrazine; 4, simazine; 5, atrazine. From ref. 130.

The on-line methodology described above allows the sample handling of rather polar analytes, such as aniline, if they are ionizable. For polar ionic organic compounds (with ionization constants below 1 or above 13), this methodology cannot be applied and their determination at very low levels is impossible.

2.2.4.3. Conclusion

We have illustrated some possibilities of on-line strategies using liquid chromatography for the sample handling of non-volatile organic compounds. This will certainly be further developed in the next few years, because of the need to monitor numerous pollutants with different chemical properties in the same analyses, to determine more polar compounds (such as pesticides and the degradation products of pollutant) and because of its potential for automation and use in field-sampling.

2.3. SAMPLE HANDLING OF VOLATILE ORGANIC COMPOUNDS

The environmental, geoscience and medical fields have an interest in monitoring volatile organic compounds (VOCs) in natural and drinking waters, which always presents a challenge for chemists. Furthermore, recommendations for safe drinking water

from the United State Environmental Protection Agency (EPA) and European Community (EC) ordinances have led to strict regulations for reducing VOC concentrations to the ppb level. These factors, in combination with the tremendous progress in GC/MS techniques during the last 10 years, have led to significant developments in the extraction of VOCs by inert gases.

The sample handling of volatile organic compounds from aqueous samples is based on gas-liquid extraction techniques. Figure 2.19 shows a schematic classification of the different methods using a gas phase for sample handling of VOCs from a liquid phase: extraction and subsequent concentration and desorption procedures.

The gas phase can be obtained by a simple thermodynamic equilibrium with the sample in a closed thermostated vessel as in static headspace methods. Volatile components are then analysed by injection of an aliquot of the gas phase into the gas chromatograph [131,132]. In dynamic headspace methods, a further preconcentration occurs by passing the gas over the sample and accumulating the sample volatiles in a cryogenic or sorbent trap.

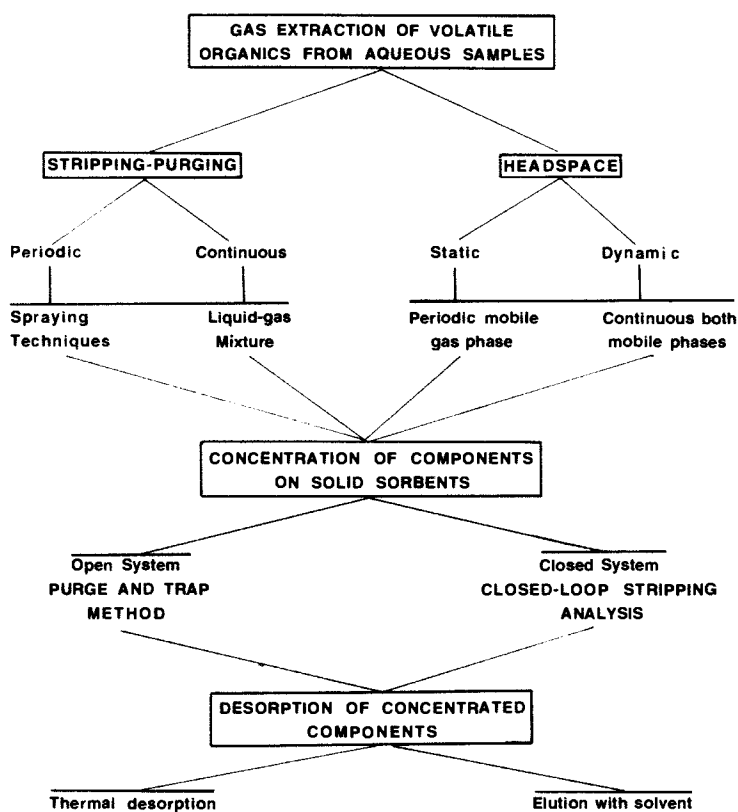


Fig. 2.19. Scheme of methods utilizing a gaseous phase for extraction and concentration of volatile organic compounds from water.

In stripping or purging methods, the gas is introduced below the surface of the aqueous samples and passes through them in the form of a stream of bubbles. The stripped VOCs are then concentrated in a trap packed with a suitable sorbent. This purge technique can be applied in a closed system (closed-loop stripping analysis or CLSA) where the gaseous phase is recycled through the sample and the trap, or in an open system (purge and trap technique or PT) where the gas strips through the sample and the trap before exhausting to the atmosphere. The trapped compounds can be desorbed by heating the sorbent trap and transferred to the gas chromatograph by an inert gas, as in the PT method. They can also be desorbed by a small volume of organic solvent which is further injected into the gas chromatograph as in CLSA techniques.

The volatile compounds can be injected off-line or on-line into a gas chromatograph. Both stripping and headspace techniques can be used for the isolation of volatile organic compounds from aqueous samples of a given volume (periodic system) or from a stream of liquid phase (continuous system).

2.3.1. Stripping-purging techniques

2.3.1.1. *Extraction*

The extraction of volatile organic compounds from water samples with gas stripping is certainly the most efficient method. To obtain a higher efficiency of extraction, it is necessary to expand the contact surface between the liquid phase and the stream of gas passing through the water sample. Several extraction procedures have been considered both for the periodic and continuous modes.

2.3.1.1.1. *Bubbling through a glass frit*

A glass tube with a medium-porosity glass frit at the bottom has been used and modified many times [133]. Purified or synthetic air, nitrogen, argon, helium and hydrogen have been used for stripping with various systems of aeration such as stirred beakers, aeration towers or spray heads.

2.3.1.1.2. *Spraying of liquid phase*

Several devices have been proposed in which the water sample is supplied under pressure to a glass vessel. It is sprayed into a nebulization cell and forms an aerosol in a stream of gas which elutes the volatile organic compounds [134]. Several variants are available, working in a periodic mode [135].

2.3.1.1.3. *Stripping method in a countercurrent packed column*

Air stripping in a countercurrent packed column has been shown to be an interesting method in the monitoring of trace organic contaminants such as trihalomethanes, chlorinated solvents and other organic volatiles [136,137].

2.3.1.2. *Preconcentration*

The concentration of VOCs is generally too low to be detected by available GC detectors. To overcome this problem, procedures for the preconcentration of volatile organics

prior to their GC analysis have been applied by trapping the extracted components from the gas phase on cold traps or solid sorbents or supports coated with a suitable stationary phase [138–140]. Trace enrichment of volatile compounds on solid sorbents is based on gas-solid extraction techniques and breakthrough can occur as in liquid-solid extractions. Breakthrough volumes depend on the nature of the sorbent (porosity, specific surface area), its amount (form and size of the trap) and on its affinity towards the analytes of interest. Many papers have been published on the determination of V_b using various solid adsorbents [141,142]. A proper choice of the adsorbent depends on the aim of the analysis. The characteristics of the analytes of interest and the physical and chemical properties of the adsorbent have to be considered [143].

2.3.1.3. Schematic apparatus features

Two procedures for the isolation and concentration of VOCs from an aqueous sample by a stream of gas and adsorption on a solid sorbent have been developed over the past 20 years. The extraction and concentration of volatiles are performed in the first case in a closed system (closed-loop stripping analysis or CLSA) [144] and in an open system in the second one (purge and trap method or PT) [145,146]. Furthermore, the desorption modes in the CLSA and PT are different; the former is performed by elution of the organics from the activated carbon with carbon disulphide, whereas in the latter, there is thermal desorption of the organic compounds from the trapping material. These differences make the CLSA and PT methods complementary for the range of organic compounds that can be analysed.

2.3.1.3.1. Closed-loop stripping analysis

Volatile components of the liquid phase are trapped in a sorbent (activated charcoal) by pumping the stripping gas in a closed circuit via the trap and the aqueous phase [144]. A schematic diagram of a CLSA apparatus is given in Fig. 2.20. At the end of the purge time (2 h), the trap is removed and then organic compounds are eluted with 8–10 μ l of carbon disulphide which is collected in a glass vial. An aliquot of this sample is subsequently analysed by GC or GC/MS. The standard addition method allows one to calculate the total mass of a component, i , in the system [147] according to the relation:

$$W_i = W_s / [(A'_i/A_i) - 1]$$

where W_i is the total mass of a component i in the whole system, W_s the mass of the standard (component i) and A'_i and A_i , respectively, are chromatographic peak areas recorded for component i in the concentrates recovered from the trap after processing the system with and without the added standard.

The standard addition method is general for calibration in the CLSA method [148]. The GC-MS identification and quantification of organic compounds in water samples has allowed considerable recent improvements in studies of pollution origins and drinking-water treatment techniques [144,149]. GC-MS computer procedures which automatically quantify purgable organic compounds, using an internal standard added to the water phase and a computer library of 215 referenced standards has been developed [150,151]. This procedure enabled the identification and quantification of 80% of the 215 reported com-

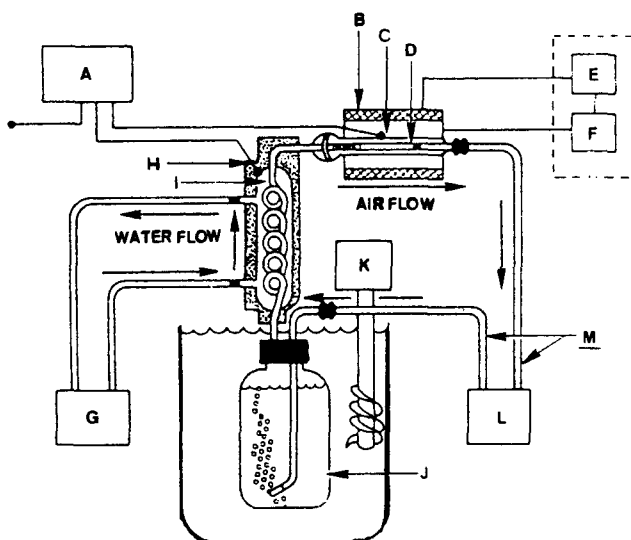


Fig. 2.20. HERL closed-loop-stripping apparatus: (A) temperature monitor; (B) heater block insulation; (C) aluminium heating block with cartridge heater; (D) glass filter holder with carbon filter; (E and F) temperature monitor (maintains 50°C); (G) thermostatically controlled water circulator (set at 95°C); (H) foam insulation; (I) glass condensing column; (J) 1-l sample bottle; (K) thermostatically controlled water bath circulator (set at 40°C); (L) metal bellows air pump; (M) 1/8-in stainless steel tubing. From ref. 168.

pounds at the 50 ng l⁻¹ level (accuracy $\pm 25\%$). The GC-MS detection limits of 1–10 ng l⁻¹ for most of the 300–400 organic compounds identifiable by this method have been reported [150–152]. A large number of volatile organic compounds [148–168] have been analysed including hydrocarbons [148,153], chlorinated hydrocarbons [153,154], substituted aromatics [144,148,153] and taste- and odour-causing compounds [155–157] in various type of aqueous matrix. These include surface and ground water [149,158, 160,161], sea water [159,160], drinking water [144,148,150,163], and waste water [164, 165]. Table 2.11 lists the advantages and drawbacks of the CLSA method.

2.3.1.3.2. Purge and trap method

The VOCs are purged from a water sample by an inert gas stream at a moderate temperature and transported to a trap [145,146]. This method, coupled to GC analyses with packed columns, is the official method employed by the US water analysis laboratories [169–174]). Several modifications of this technique have been made relating to the adsorbent and the design and size of the device. Sorbent traps packed with Tenax and numerous composite sorbents (Tenax-Silicagel, Tenax-GC-activated carbon, Tenax GC-Chromosorb, Chromosorb, Carbosieve S-III-Carbopack B) are commonly used to concentrate the VOCs from the purge gas [175 and refs. therein]. Cold trapping can also used with PT techniques, especially when open tubular columns are used for the chroma-

tographic analysis. Cold trapping has the advantage that thermally labile and polar compounds are less affected during the trapping and the thermal desorption [8].

Many semi-automatic instruments have been described [176–179]. Several designs equipped for on-line detection are available [177,180]. Figure 2.21 shows a schematic view of a commercial automated purge and trap system [181]. The direct interfacing is accomplished without splitting of the purge and trap desorption carrier flow. The whole process (sampling, purging, trapping, injection and analysis) is automated and takes 70 min. With aqueous sample concentrations ranging from 0.1 to 10 $\mu\text{g l}^{-1}$, recoveries are close to 100% and analyses are currently performed on 10-ml samples. With few exceptions, the detection limits range from 10 to 90 ng l^{-1} . Accurate determinations have been reported of VOCs in drinking water, at concentration levels ranging from 0.2 to 2 $\mu\text{g l}^{-1}$, using a standard inert gas purge-extraction and isolation of the VOCs on a three-stage solid-phase trap. Analysis of the components on capillary GC coupled to an ion trap detector ensured identification and concentration measurement of 54 analytes with an automated system [182].

TABLE 2.11

COMPARISON BETWEEN THE CHARACTERISTICS OF CLOSED-LOOP STRIPPING ANALYSIS AND THE PURGE AND TRAP METHOD

Advantages	Drawbacks
Closed-loop stripping analysis	
– Ultra-sensitivity (1–10 ng l^{-1})	– Limited range of purgable compounds
– Relatively trouble-free	– Low recoveries for highly volatile compounds
– Blanks are rather clean	– Moderately and highly polar ionizable organics are poorly purged or not recovered
– Rapidity (elution of VOCs from the activated carbon trap requires 10 min)	– Use of highly toxic solvent (CS_2)
	– Very polluted samples may overload the activated carbon trap and contaminates the closed-loop
	– Careful and consistent manipulations
Purge and trap method	
– Wide range of application (even for semi-volatile organics)	– Risk of cross-contamination in the purging vessel when high and low concentration samples alternate
– Wide survey of identifiable volatile organics in drinking water	– Risk of sample foaming
– Good accuracy	– Requires ultrapure gas
– No organic solvent elimination step	– Possibility of losses of very volatile compounds
– Easy coupling with cryogenic traps	– Risk of degradation of thermally unstable compounds
– Possibility of on-line coupling with a suitable analyser (GC and GC-MS)	– Risks of bleeding from the sorbent bed
– Easy automation	

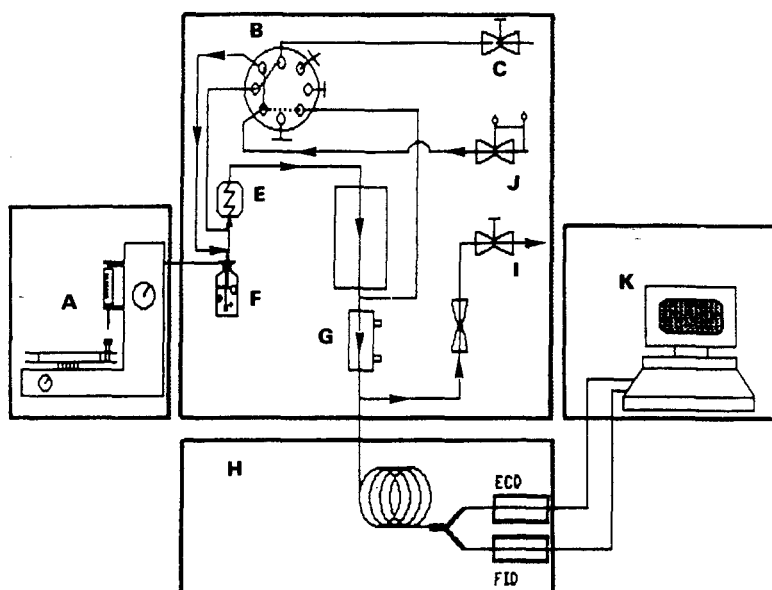


Fig. 2.21. Schematic view of the purge-and-trap system. See valve for (—) purge mode and (-----) desorption mode. (A) Sampler; (B) Chromack PT injector; (C) backflush; (E) condenser; (F) purge vessel; (G) trap; (H) gas chromatograph; (I) vent; (J) carrier; (K) computer. From ref. 181.

As can be seen in Table 2.11, the advantage of PT techniques is their wide range of application and their potential for on-line coupling with GC analysis, with easy automation.

2.3.2. Headspace analysis

2.3.2.1. Static headspace analysis

This method determines the volatile components in a liquid or a solid matrix by analysing the vapour phase that is in thermodynamic equilibrium with the sample in a closed system [131]. The sample is placed in a glass vial and closed with a rubber septum [183–185]. The vial is thermostated until equilibrium is established. The gas phase is sampled manually by a gas-tight syringe or with an electropneumatic sampling system in automated headspace analysers [186,187]. These automated systems are designed to be interfaced very easily with some chromatographs (DANI HSS 3950) [188] and (Perkin-Elmer HS-100 Automatic Headspace Samplers) represented in Fig. 2.22 [189].

With the automated analyser, a needle enters the sample vial through the rubber septum and the gas phase is pressured up to the column head pressure (Fig. 2.22A). Then a valve switches off the carrier gas flow and the excess pressure is automatically released. The volatile components are simultaneously carried into the GC column (Fig. 2.22B). At

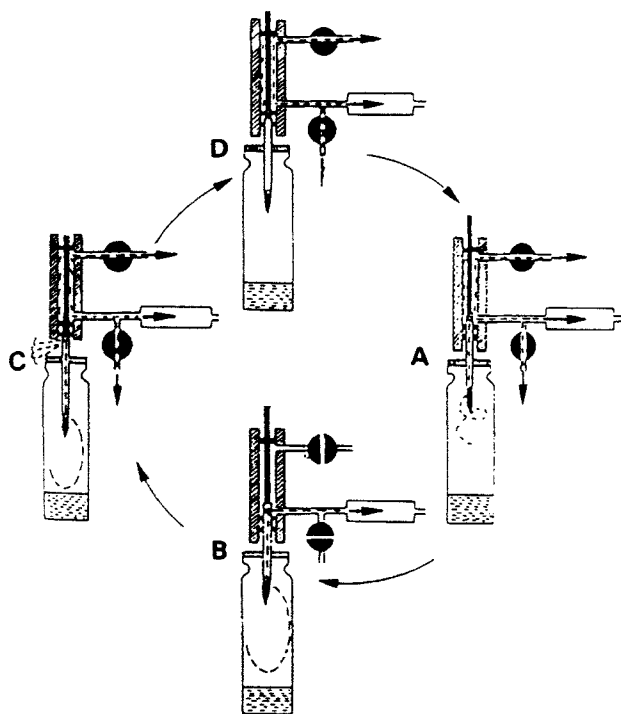


Fig. 2.22. Multiple headspace extractions: Perkin-Elmer HS-100 automatic headspace sampler: (A) pressurization; (B) sampling; (C) vent; (D) standby. From ref. 189.

this point, the valve of the automatic injector closes and the carrier gas supply is restored (Fig. 2.22C,D).

Several fully automated headspace samplers have been proposed to analyse up to a 100 samples. A static headspace method has been used for the determination of numerous compounds in various types of aqueous matrix: trace amounts of sulphur compounds [190], nitrous oxide [191] and organohalogen compounds generated during chlorination of water [192–195]. The headspace-gas chromatography technique coupled to electron capture detector (ECD) allowed the determination of organohalogens at the picogram per litre level [196].

Nevertheless, the application of the static headspace technique to volatile trace analysis has several limitations; solutes at very low concentration are often undetectable, as are low vapour-pressure compounds. Complex mixtures of VOCs, e.g. odour- and flavour-causing compounds, need to be analysed with high resolution capillary columns which have a low injection capacity that affects the detection limit. For these reasons, dynamic headspace procedures have been developed.

Table 2.12 reports the advantages and drawbacks of headspace techniques. The main advantages of the static headspace technique are its simplicity and the absence of artifact and related troubles, which makes it interesting for the handling of complex samples. The main drawback is its limited sensitivity for low vapour-pressure compounds.

TABLE 2.12

CHARACTERISTICS OF STATIC HEADSPACE METHOD

Advantages	Drawbacks
<ul style="list-style-type: none"> – The simplest method – Requires small volume samples – Artifact and trouble free – Relatively accurate quantification of major components – Possibility of studying equilibria – Easy fingerprint for quality control – Easy automation 	<ul style="list-style-type: none"> – Low-vapour pressure compounds often undetectable – Very complex mixtures require GC columns with low sampling capacity – Less performance in trace analysis than CLSA or PT methods

2.3.2.2. Dynamic headspace analysis

In dynamic headspace techniques, the headspace vapours above the sample are continuously removed by means of a gas flow, often with subsequent concentration of the vapour components on cold or sorbent traps [175]. This method is less efficient than PT or CLSA methods whose efficiency has been improved by passing the gas through the solution.

2.3.3. Conclusion

Stripping-purging or headspace procedures are very efficient for the extraction of volatile compounds from water samples. With a coupled concentration step as in PT and CLSA methods, their determination at very low levels can be obtained easily and automatically. The choice of method depends on the physico-chemical parameters that determine the distribution between the gas and liquid phases.

2.4. CONCLUSION AND FURTHER DEVELOPMENTS

As new ways of increasing the speed and efficiency of sample preparation processes are based on automated on-line strategies, there is a particular interest in carrying out selective SPE and clean-up procedures. Emphasis has been given to this topic in this chapter. We have also pointed out the need for multi-residue methods to allow us determine organic compounds over a wide range of polarity including the more polar ones. The development of LC in environmental analysis and its coupling to MS detection will certainly help in the adoption of on-line techniques. It is then expected that field sampling on small cartridges will become more popular and that more applications using selective SPE techniques will be developed in the environmental area.

Some aspects of sample handling have not been discussed in this chapter either because they are very specific towards some special compounds or because they are still not

fully developed; for example, the enrichment techniques using supported liquid membranes. On-line prederivatization has not been discussed but can help for the preconcentration of some polar analytes.

Sample handling will certainly remain the object of new developments in the future. The potential of on-line SPE can be enhanced by developing more prepacked precolumns to increase the choice of the sorbents, and by developing selective sorbents such as metal-loaded phases, selective ion-exchangers, phases containing immobilized enzymes and novel sorbents for polar and water-soluble compounds.

Future studies on sample preparation will certainly also concentrate on improving hyphenated techniques such as capillary zone electrophoresis-LC, micro SPE-GC, micro SPE-microbore LC or SPE-LC-LC. But one must bear in mind that the sample preparation cannot be regarded as a separate step and that coupling the sample-handling techniques with selective detection modes can both simplify them and provide a more powerful analytical procedure.

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Chapter 3

Extraction, clean-up and recoveries of persistent trace organic contaminants¹ from sediment and biota samples

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¹The term "contaminant" has been used rather than "pollutant" since the latter should be reserved for those compounds that have a known biological effect. In this chapter, the analysis of compounds which come under both categories are discussed without distinction.

3.1. INTRODUCTION

The power of analytical instrumentation currently available makes it possible to detect toxic organic contaminants at concentrations below 10^{-12} in environmental samples. Such low detection limits are essential if these contaminants are to be measured with the accuracy and precision required for correlation with biological effects data. In turn, these detection requirements place additional demands on the sample extraction, clean-up and group separation schemes particularly as more chemicals are added to the list of determinands [1]. Master analytical schemes [2–4] have been used to great effect for the determination of specific contaminants in water, e.g. Environmental Protection Agency (EPA) Priority Pollutants Protocols. However, the diversity of sediment and biological matrices sometimes makes any single analytical scheme for the analysis of trace organic contaminants more difficult to devise and to validate [5–7].

The environmental analytical chemist is placed somewhat in a dilemma. The cost of sampling, and, in many cases, the irreplaceable nature of the samples makes it imperative to obtain as much chemical information as possible from the material collected. Inevitably, samples will be obtained for specific chemical measurements, but in addition to the primary objectives, other qualitative information can prove invaluable. However, the wide angle analytical approach, which continues to add compounds to a single scheme, will ultimately lead to conflicting methodological requirements and an unacceptable level of compromise. As a result, two types of sample preparation schemes have emerged which reflect the extremes of this approach. Firstly, the highly specific method, for the determination of a single or very small group of similar compounds, and secondly, multi-residue schemes which cover the preparation and separation of the maximum number of compounds possible for broadly based chemical information [1,8].

The isolation of the determinand(s) from both the matrix (extraction) and other bulk and trace organics (clean-up) must be fully optimized and highly efficient. Apart from instrumental calibration, the analytical variability of any gas chromatographic (GC) or GC-mass spectrometric (GC-MS) determination of trace organics is primarily caused by interference from non-target compounds, which have not been removed from the extract. Increasing the specificity of the detector does not necessarily remove the problem, but merely serves to hide the direct evidence of the interference. Varying amounts of extrac-tants which co-elute with the determinand will affect the detector signal giving rise to a reduced or even negative response [9,10]. Improved reliability and robustness of a method is more likely achieved by efficient sample preparation than by some form of screening by a selective detector.

This chapter discusses the application of relatively new techniques, particularly super-critical fluid extraction, in relation to their value as an extraction method compared with other established procedures, such as Soxhlet extraction. The rising cost of sample preparation and the need to measure contaminants at the ultratrace level requires the introduction of automation and on-line systems into routine analysis. These on-line systems are contrasted with the requirements of multi-residue analysis schemes. The efficacy of any technique is dependent on the recovery of the determinand from the matrix. The principles and practices of estimating the recovery of trace contaminants and values obtained in the various studies are discussed.

3.2. RECOVERY

3.2.1. Principles of recovery measurements

Recovery measurements are one of the more difficult and ill-defined aspects of trace organic analysis. These measurements are often completed, with the minimum number of replicate determinations over a limited concentration range, to optimistically justify the use of a method. Experiments designed to obtain the efficiency of the analytical method often implicitly assume that this also includes the efficiency of extraction from the matrix. The term *recovery* is generally misinterpreted and many papers which report recovery data from sediment and biota can serve to prolong this problem.

The basic requirement is to estimate how much of the determinand has been removed from the natural matrix by a given extraction technique. However, the widespread practice of simply adding a known amount of the determinand to the matrix, usually in an organic solvent, prior to extraction and subsequent analysis, does not answer this question. This type of spiked sample analysis will determine the accuracy and precision of the subsequent analytical steps, but does not necessarily measure the efficiency of extraction.

To determine the efficiency of extraction, it is imperative that the contaminant is bound to the matrix in a similar configuration to that which exists in the environment. The extraction efficiency can then be measured for that determinand in a specific matrix configuration.

At present, water is the only matrix where this can be achieved in a relatively straightforward way. The determinands are added below the surface of the sample in a small ca. 1–2 ml volume of water miscible solvent. The water must be completely mixed and allowed to stand at least overnight prior to extraction to allow the contaminants to come into equilibrium with the other organic materials, particularly humic materials. The spiked water sample must be analysed in its entirety, including the inner surfaces of the container, either separately or as a single determination.

Wet sediments can be dosed with known amounts of the determinand by adding the contaminants in a small volume ca. 2 ml of water-miscible solvent such as acetone, to the sample and the interstitial water. The sediment and pore water are mixed thoroughly in a closed container for not less than 24 h and then allowed to settle for a further 24-h period prior to a final mix. The sediment can be subsequently freeze dried if non-volatile determinands are required, but for more volatile determinands, e.g. chlorobenzenes, the sediment should be drained of any excess water and extracted as a wet sediment. The filtered pore water should also be analysed. If the organics are mixed completely with the sediment and are given sufficient time to adsorb and diffuse into the sediment surface, then most lipophilic, hydrophobic determinands with an adsorption coefficient of $>10^2$ will be almost completely associated with the organic fraction in the sediment [11]. The sample should be analysed in its entirety to reduce any errors associated with the heterogeneity of the sample.

Organic contaminants can only be fully bound into biological tissue through feeding or exposure studies. The animal distributes the trace organics throughout the body, partitioning and possibly metabolising the material in the normal way. The dose can also contain a small fraction of a radiolabelled tracer, e.g. ^{14}C which could be measured directly in a

specific tissue after sacrificing the animal. The concentration in the tissue from the scintillation measurement of the radiolabelled material can be compared to the amount determined by organic extraction, clean-up and analysis by GC or GC-MS to determine the full efficiency of the method. Such experiments are extremely costly and time-consuming and can rarely be justified on the basis of determining extraction efficiency alone.

Alternative methods are less expensive, but should not be regarded as a measure of extraction efficiency per se. Where it is not possible to obtain an absolute comparison with a spiked value, it is necessary to select a method which gives the highest recovery of the determinand from the natural matrix using an exhaustive or comparative technique. Unspiked tissue samples are extracted sequentially by the same method to determine the time required for the maximum removal of the contaminants. Normally, the time period for each set of conditions, e.g. solvent or temperature/pressure supercritical fluid extraction (SFE) is extended until the subsequent extraction contains none of the analyte(s). Direct comparison of different methods or the same technique with different conditions can be made. Unfortunately, prior to the emergence of SFE, only a few detailed comparisons [12] have been made.

3.2.2. Direct measurements

The efficiency of the extraction of compounds from solid matrices using the established techniques can be compared with an *in situ* measurement. Lai et al. [13] have used supersonic jet laser-induced fluorescence spectroscopy (SSJ/LIF) to determine PAHs in sediments. The essential elements of the SSJ/LIF are: (i) a pressurized sample chamber where the solid sample is heated; (ii) a nozzle connecting the sample chamber to the fluorescence cell; (iii) an evacuated fluorescence chamber through which the laser beam is passed. The sediments were heated to 200°C to produce the PAH vapour. The LIF signal appeared within 20 s and persisted for 5–30 min. By selecting the correct monitoring wavelength, 386.74 nm for benzo[a]pyrene and 367.44 nm for pyrene, it was possible to distinguish between the two PAHs. Quantitative analyses were carried out by alternating the standards and unknown samples and comparing the integrals of the LIF signal. This technique is both precise and accurate with the limit of determination of 900 ng for benzo[a]pyrene and 200 ng for pyrene in the solid sediment.

3.2.3. Recovery measurements in practice

In the initial stages of any method development, recovery measurements are made to optimize the conditions of extraction and sample treatment. It is difficult to be categorical about the percentage level of recovery which is regarded as acceptable for a method. Clearly, some workers accept values of <60%. Where methods give a recovery ca. <75% it is essential to determine whether this low value is dependent on the specific type of matrix structure, e.g. percentage organic carbon in the sediment or lipid in tissue. Methods with such a low recovery are also likely to have a greater variance associated with the measurement. In all cases there should be an estimate of the recovery for the batch of samples being analysed. Data on recovery are often limited to a few initial measurements

(ca. 1–5) at a single or, at the most, two different levels of concentration, which is an inadequate basis to make any further corrections to the final data. Since the only purpose for making recovery measurements is to be able to apply a correction factor to the data subsequently produced, it is vital to have a good estimate of the errors associated with that recovery. Where the recovery is variable and sufficiently low to warrant the data being corrected, then this recovery measurement must be made at the time of the analysis. It is insufficient to use recovery information obtained during the initial validation of the method to correct measurements made some considerable time afterwards.

Regular, routine sample recovery measurements² can be made using the method of standard addition. The matrix is spiked with the determinands in a small volume of solvent at a level which is ca. 50%, 100%, 150% and 200% above the estimated level in the sample. A number of independent replicates should be made at each level. Provided that sufficient material is available the sample can be analysed prior to spiking. In case of limited size, e.g. small fish livers, a number of samples may be pooled and homogenized for such recovery experiments.

Standard addition to wet sediment should be made in a water-miscible solvent e.g. acetone or methanol. Any convenient solvent can be used to spike dry sediment. Standard addition to tissue samples can be made by first spiking a small amount of silica and allowing the solvent to evaporate. The silica is then ground with the tissue prior to extraction.

Following the analysis of the spiked samples, the data are plotted to determine the average recovery and the confidence interval of the method (Fig. 3.1). Once this recovery is established then a single or duplicate recovery sample can be analysed at regular intervals to check the validity of the regression. In this way a series of data are obtained over a period of time to give a long term estimate for the efficiency of the method.

Isotope dilution mass spectrometry (IDMS) is a preferable and more elegant method to overcome the whole problem of sample recovery [14,15,17]. The ¹³C-labelled isotope of the determinand is added to the sample at the commencement of the analysis and the ratio of the labelled and unlabelled compounds are measured by MS. This technique eliminates the need for recovery measurements and automatically accounts for any losses in the determination. The two main limitations of this method are the cost and availability of the labelled compounds and the need to use the MS as a detector.

3.3. PRE-EXTRACTION TREATMENT

Samples of sediment and tissue collected in the field are usually preserved by freezing immediately, either on board ship or at the laboratory. Rapid preservation is vital if the integrity of the sample is to be maintained. Sediment cores should be sectioned and each sub-sample individually frozen in liquid nitrogen. Some core samplers allow the whole core to be frozen in situ prior to sectioning. This technique is preferable, if these facilities are available, since it allows the top interstitial sections to be handled more easily.

²Sample recovery measurements are a measure of the efficiency of the analytical method and do not include a measure of the extraction efficiency, as explained above.

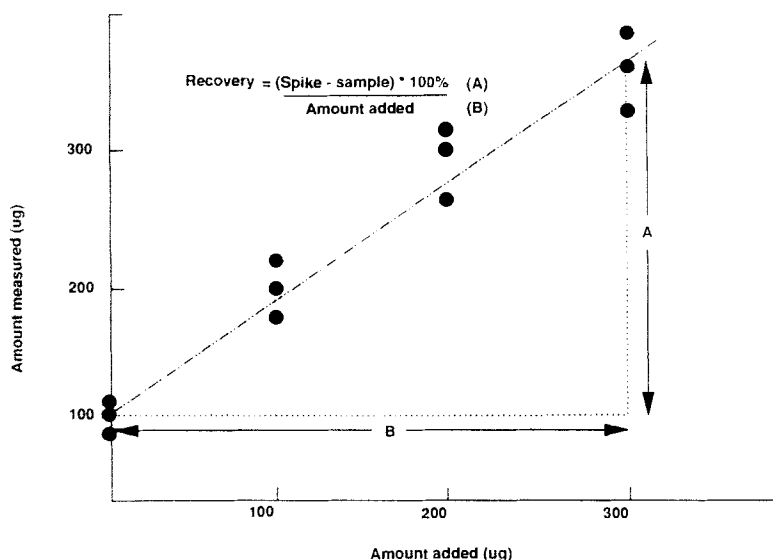


Fig. 1. Measurement of recovery of organics using matrix matching. Additional replicate samples are spiked with the determinand up to five times the estimated level. A measure of the recovery is made from the regression analysis of these data.

Wherever possible, the fish or animal should be dissected immediately and the individual tissue frozen in liquid nitrogen, rather than using the normal deep freeze which may take several hours to preserve the material [17]. The tissue should be stored in individual packs of approximately the size required for analysis to minimize sub-sampling thawed material.

Sediments and soils can be treated in different ways prior to extraction depending on the purpose of the programme [18]. Sediments are more conveniently stored as dried powders. However, this technique is not appropriate if relatively volatile contaminants such as 1-ring aryl hydrocarbons (e.g. alkyl benzenes, chlorohydrocarbons, chlorobenzenes), 2-ring PAHs, (e.g. naphthalene) are to be determined. In such cases, the sediment should remain frozen prior to analysis and extracted as the wet material.

Most trace organic contaminants are associated with the organic fraction of the sediment, since they partition into the lipids and waxes on the sediment surface. The large proportion of the total organic carbon (TOC) is usually associated with finer particles and an arbitrary value of $63 \mu\text{m}$ has been selected to isolate the organic fraction of the sediment [19]. When this fraction is required for a separate analysis, it is advisable to wet sieve the sample, since dried sediments must be re-ground to break up the agglomerates. Re-grinding does not produce the original particle size distribution of the sediment or soil.

The sieved samples which are to be analysed for the less volatile components can be freeze-dried or air-dried at ca $35\text{--}40^\circ\text{C}$. The resultant sediment brick will require gentle grinding to obtain a free flowing powder.

3.4. EXTRACTION

Although selective extraction of organics appear to be an attractive option, the different types of adsorption sites on soils and sediments or binding sites in tissues, requires an exhaustive technique to recover the maximum amount of the determinand from the substrate. This is particularly true where the chemicals to be extracted cover a broad range of polarity, reactivity and molecular size. Extraction, therefore, is primarily a process of separating all of the determinands as completely as possible from the bulk of the matrix. This process will inevitably remove unwanted co-extracted materials as well. Selective extraction only becomes a possibility when a small number of chemically similar compounds are to be isolated.

3.4.1. Supercritical fluid extraction (SFE)

The first use of SFE as an extraction technique was reported by Zosel [20]. Since then there have been many reports on the use of SFE to extract chlorobiphenyls (CBs), polynuclear aromatic hydrocarbons (PAHs) and other organics from particulates, river sediment and plant tissue [21–28]. The attraction of SFE as an extraction technique is directly related to the unique properties of the supercritical fluid [29]. Those fluids which have been used have a low viscosity, high diffusion coefficients and low flammability, all of which are clearly superior to the organic solvents normally used. Carbon dioxide is the most common supercritical fluid to be used, since it is inexpensive and has a low critical temperature (31.3°C) and pressure (72.2 atm). Other less commonly used fluids include nitrous oxide, ammonia, fluoroform, methane, pentane, ethanol, sulphur hexafluoride and dichlorofluoromethane. Most of these fluids are clearly less attractive as solvents in terms of toxicity or as environmental friendly chemicals. A summary of recent SFE applications is given in Table 3.1. Commercial SFE systems are available, but some workers have also made inexpensive modular systems [30].

Levy et al. [31] made a brief investigation of alternative fluids for on-line SFE-capillary GC with carbon dioxide (CO₂), nitrous oxide (N₂O), and sulphur hexafluoride (SF₆) for the extraction of PAHs and alkanes from waste sediment, sediment and shale rock. They initially compared the extraction efficiency of pure fluids and then some fluid mixtures. They found that 20% SF₆ in CO₂ was more effective at 375 atm, 50°C for 30 min than the pure fluid for removing both PAHs and alkanes.

McNally and Wheeler [32,33] applied SFE to the analysis of sulphonylurea herbicides and their metabolites in soil and plant material. Engelhardt and Gross [34] analysed aldrin, lindane (α -hexachlorocyclohexane) and 4,4'-DDT in spiked soil samples using SFE followed by supercritical fluid chromatography. Lopez-Avila et al. [36] used SFE to extract a series of 17 organochlorine pesticides and 25 organophosphorus pesticides from sand using CO₂ and CO₂ modified with acetone. The same group [36] reported the extraction and recoveries of 41 organochlorine and 47 organophosphorus pesticides extracted from sand over a range of temperatures and pressures. The pressure in the extraction vessel (100 mm \times 10 mm i.d.) was maintained by using a 600 mm \times 0.05 mm i.d. fused silica restrictor and the CO₂ extract collected in solvent spiked with terphenyl-*d*₁₄. Hexane was used as the collecting solvent for the CO₂ experiments, and dichloromethane

TABLE 3.1

METHODS FOR THE EXTRACTION OF TRACE ORGANIC CONTAMINANTS IN SEDIMENT AND BIOTA SUPERCRITICAL FLUID EXTRACTION

Application	Extractant	Conditions	Reference
CBs, PAHs, nitro PAHs soil, particulate and sediment	CO ₂	345 atm at 40°C	Schantz and Chester [21]
CBs, DDT, toxaphene in top soil	CO ₂	100 atm at 40°C for 10 min	Brady et al. [114]
PAHs in river sediment	CO ₂ 5% methanol N ₂ O	Various	Hawthorne and Miller [23]
TCDD in sediment	2% Methanol in CO ₂ and N ₂ O	310 atm at 40°C for 30 min	Onuska and Terry [66]
Chlorpyrifos in wheat	CO ₂	100–400 atm at 25 atm min ⁻¹	Campbell et al. [65]
PAHs in sandy loam soil	3% Methanol in CO ₂	Various	Andrews et al. [115]
Organochlorine pesticides and CBs in soils and sediments	CO ₂ 5% co-solvents toluene, acetone, methanol, acetic acid diethylamine	100 atm at 40°C	Dooley et al. [67]
Chlorinated pesticides and CBs in biological tissue	CO ₂	100 atm at 50°C to 150 atm	Nam et al. [39]
Chlorinated pesticides and CBs in blood and milk	CO ₂	160 atm at 50°C	Nam et al. [38]
Triazine herbicides in process waste from the dairy industry	CO ₂	150 atm at 50°C	Nam et al. [38]
n-Alkanes and PAHs in fish PCDDs in liver	CO ₂	170 atm at 50°C	Nam et al. [38]
Coal tar contaminated soil	5% Methanol in CO ₂	23 atm at 32°C for 7 h	Yu et al. [69]
Sterols in hamster faeces	CO ₂	400 atm at 100°C for 1 h	Pinkston et al. [71]
Organochlorines and PAHs in soils	CO ₂	Various solutes trapped on solid absorbents	Lohleit and Bächmann [70]
Chlorinated pesticides PCBs in biological tissue and chlorophenols in wood	CO ₂	170 atm at 50°C	Kapila et al. [41]

or methanol was used with CO₂ modified with methanol (9:1). They also extracted two reference materials (RMs) certified for 17 PAHs. Initially the recoveries for PAHs in one RM (SRM HS-3) were very low (6.2 and 69%) when CO₂ was used alone. Most recoveries were <50% and the recovery of four PAHs was <20%. The efficiency of extraction increased to between 28 and 93% with the addition of 10% methanol as a modifier. Only four of the 16 PAHs had recoveries measuring <50%. The recoveries for the second RM SRS 103–100 ranged between 23 and 107%. Of the 15 PAHs, eight were <50%. The study looked at the effects of seven variables on the PAH recovery. The most influential parameters were the extraction time and pressure, followed by moisture content and sample size. An estimate of the relative cost for SFE and Soxhlet extraction, including capital, consumables and staff showed that SFE was between 2.5 and 3 times more expensive per sample.

Nam et al. [37,38] attempted to selectively extract a wide range of xenobiotics from biological tissue without removing interfering, co-extracted materials and hence overcome the need for additional clean-up steps prior to GC analysis. They used lower pressures and temperatures to attempt this selective extraction. Blood and milk were adsorbed onto florisil and dried with anhydrous sodium sulphate. The double extraction was flushed into hexane and the concentrate determined directly by GC. In this case, the biological fluids were spiked at levels between 1 and 20 $\mu\text{g kg}^{-1}$. They achieved sufficient selectivity in the extraction to avoid the need for further clean-up. The double extraction for 2 \times 1 h demonstrated that (i) the single extraction was sufficient and (ii) the quality of the “commercial grade” CO₂ was not sufficiently pure for this application. The same workers [39] extracted between 93 and 104% of the CBs and organochlorine pesticides (OCPs) from biological tissue at 142 atm and 50°C when the extraction vessel was left to equilibrate for 1 h.

Fish tissue spiked with *n*-alkanes and PAHs were recovered with SFE CO₂ [38]. By reducing the solvation power of the CO₂ less than 10% of the total lipid was removed with over 80% recovery of the alkanes. However, pressures in excess of 200 atm were necessary to recover PAHs with >3-rings. Between 73 and 95% of the thiophosphate pesticides and phenoxyester herbicides were recovered, but the more polar triazines herbicides and carbamate pesticides were not so soluble in the CO₂ (46–86%). Approximately 70% TCDD was recovered from animal tissue spiked at levels between 0.05 and 1 $\mu\text{g kg}^{-1}$ using CO₂ with toluene as an entrainer. In general, extractions with CO₂ can be predicted to give recoveries of >75% for organic molecules with an octanol/water partition coefficient (K_{OW}) ≥ 2.5 [40].

Kapila et al. [41] reviewed their studies in SFE of OCPs from biological tissue. Recoveries from fish, milk and blood samples were $\geq 95\%$ for a range of OCPs. This was primarily feasible when the extraction time was sufficiently long, ca. 1 h at 175 atm and 50°C. They also used an “on-line” system for the determination of chlorophenols in wood chip and pigments. The recoveries of the chlorophenols were between 75 and 104% with a limit of determination of 1–4 mg kg^{-1} and a precision of $\pm 2\%$. The sample intake mass was 15 g.

Some improvement in the level of selectivity in SFE of biological tissue has been made by the addition of solid adsorbents to the tissue in order to bind the lipids while the contaminants are extracted. Johansen et al. [42] ground fish tissue with anhydrous sodium

sulphate and basic alumina prior to extracting with CO₂ at a fluid density of 0.57 g cm⁻³. Recoveries ranged between 70 and 86%. The extracts were cryofocused prior to re-injection by thermal desorption with little or no interference from any lipid. This method of extraction gives promising evidence of improved selectivity, but interferences from other co-extracted materials still require further separation in most samples prior to the final determination.

3.4.2. Soxhlet extraction

Soxhlet extraction has been used for the isolation of non-polar and semi-polar trace organics from a wide variety of sediments, soils, animal and plant tissues [43–47]. The size of the systems can vary, but the more common configurations use between 100 and 200 ml of solvent to extract between 20 and 200 g of sediment and 1 and 100 g of biological tissue³. Larger systems can be used, but require proportionally more solvent. It is essential to match the solvent polarity to the solute solubility and to thoroughly wet the matrix with the solvent when extraction commences. Animal and fish tissue are first macerated and then ground with sodium sulphate and silica. This grinding reduces the water content and helps to open up the tissue structure.

Non-polar solvents such as *n*-hexane have been used to extract non-polar contaminants such as OCPs and CBs. While these solvents are relatively efficient for removing organochlorine from fatty tissue that has have a predominance of triglycerides, they are not completely extracted from low fat tissue. De Boer [12] made a comparative study of the extraction efficiency of different solvents for CBs in fatty and lean fish tissues. The comparison was made between pike perch, perch, bream, roach and eel and the solvents were *n*-pentane, *n*-pentane/diethyl ether (1:1), dichloromethane and acetone/*n*-hexane (1:9). He also compared these extractions with the saponification of the tissue with 40% potassium hydroxide in ethanol (1:1) at 90°C for 4 h prior to extraction. The main conclusions of this work were that samples should be left for a minimum of 2 h to dry completely after grinding with sodium sulphate. A longer time was unnecessary. Extraction with non-polar solvents such as *n*-alkanes took considerably longer (>6 h) and were not as effective as polar solvents such as dichloromethane in removing the CBs and the lipid. This was less evident for fatty (triglyceride) tissue, reflecting the relative distribution of CBs bound onto the phospholipids and the partition into the neutral lipids. A maximum of 6 h extraction with a polar solvent was recommended. Although higher recoveries were obtained by initially saponifying, van der Valk and Dao [48] found that prolongation of the hydrolysis reaction at temperatures above 70°C and >1 h resulted in a loss of CB180. Wells and Echarrri [47] also found that the highly chlorinated CBs were also dechlorinated and hydrolyzed.

Sediments and soils need to be thoroughly wetted to obtain an efficient extraction. Surface tension of the solvent across the pores of a dry sediment are sufficient to prevent complete diffusion of the liquid into the micro-cavities of the sediment. Non-polar solvents do not readily wet the surface of dry sediments and are too immiscible with water to

³The mass of biological tissue is usually less than sediment since it is ground with sodium sulphate prior to extraction, reducing the overall tissue mass which can be placed in the Soxhlet.

be able to penetrate the wet material. This problem can largely be overcome by: (i) damping the sediment with an electrolyte, e.g. 1% ammonium chloride overnight; (ii) using a binary mixture such as acetone/hexane or dichloromethane which has sufficient polarity and water solubility to wet the surface. In view of the need to remove the waxes and lipids after extraction of the sediment, some workers have saponified the sediment prior to extraction. In some cases, this technique can result in an even higher recovery [49].

3.4.3. Blending and ultrasonic extraction

The simplest extraction technique is to blend or ultrasonicate the sample with an appropriate organic solvent at room temperature. Apart from the polarity of the solvent, the efficiency of the extraction is dependent upon the homogeneity of the biological tissue and the mixing-ultrasonication-blending-soaking time. The mixture of sample and organic solvent are separated from each other by filtration and washing with solvent.

Blending has been used for soils, sediments and milk [50,51] plants, animal tissue, fish and shellfish [8,52]. Jensen et al. [52] and Jansson et al. [8] used an Ultra Turrax blender and solvent extraction in a separating funnel for a wide range of polychlorinated and polybrominated contaminants in fish and animal tissue. Wahlberg et al. [53] isolated nonyl phenols and nonyl phenol ethoxylates from biota. The tissue was homogenized with *n*-hexane/acetone (1:2.5) in a separating funnel and allowed to stand for 5 min, after which the mixture was filtered into a second funnel containing 0.9% sodium hydroxide and phosphoric acid. The tissue was washed with a further two portions of diethyl ether in hexane (1:10). The determinands were isolated in the hexane layer in the lower separation funnel.

Stanley et al. [54] extracted polychlorinated diphenyl ethers from human adipose tissue by blending it with dichloromethane after homogenizing the tissue. Larsson et al. [55] studied several groups of mutagens including PAHs, nitro-PAHs and oxy-PAHs in grilled and smoked fish. The samples (500 g) were homogenized in acetone (500 ml) to obtain both the basic and the neutral/acidic fractions. The mixture was vacuum filtered and the solids were re-homogenized and extracted. The combined filtrates were cooled to -55°C and filtered to partially remove most of the fat and protein. The filtrate was evaporated to near dryness and reconstituted in hexane. Bolygó and Hadfield [56] extracted synthetic pyrethroid residues from fish eggs by homogenization and hot acetonitrile extraction in a sealed vial at 80°C for 1 h.

Holstege et al. [57] developed a multi-residue screen for the quantitative determination of 43 organophosphorus insecticides in both plant and animals. The plants were fractured in a Stein mill with liquid nitrogen and the animal tissue prepared in a Waring blender. The samples were homogenized with sodium sulphate and methanol/dichloromethane for 2 min. Excess water was removed with additional sodium sulphate. The mixture was centrifuged at 1200 rev./min for 5 min and the centrifugate evaporated to almost dryness using decanol as a keeper.

The extraction of chloroaromatics such as chlorophenols, chloroguaiacols and chlorocatechols is complicated by the different sorption processes that control the binding within the soil-sediment structure [58–60]. The free, physically adsorbed chlorophenolics can be extracted with solvent, but this may only account for between 1 and 5% of the total

concentration of these contaminants in the sediment. Martinsen et al. [61] found that *n*-hexane or cyclohexane and iso-propanol recovered <1% of the tri- and tetra-catechols in sediment. Remberger et al. [62] attempted to extract both the "free" and the "bound" fractions with acetonitrile/hexane/methyl tert-butyl ether solvent mixture. However, a higher recovery (25–100%) was obtained by using methanolic potassium hydroxide. Wells et al. [49] reported the same improvement with saponification for the recovery of some CBs from sewage sludge during an intercomparison exercise. Brezney and Joyce [60] made a comparative study of the recovery of 10 hydroxychloroaromatics from soils using conventional solvent extraction and in situ acetylation. The soils (20 g) were spiked with a stock solution in methanol/water (1:1), homogenized and left for 18 h. Five different extraction methods were compared: (i) sonication of sodium sulphate dried soil with dichloromethane; (ii) similar sonication with ethyl acetate; (iii) soaking with acetonitrile and ascorbic acid; the soil was left overnight treated with dilute sulphuric acid and extracted with methyl tert-butyl ether; (iv) sonication with ethyl acetate, acetic anhydride and pyridine; (v) acetylation with acetic anhydride in pyridine followed by sonication with ethyl acetate. Some of the hydroxy phenolics gave good recoveries for all methods, e.g. 4,5-dichloroguaiacol, 3,4,5-trichloroveratrol, tetrachloroguaiacol and tetrachloroveratrol (80–99%) while others like 4,5-dichlorocatechol (1.3–59%) and 4,6-dichloroguaiacol (49–74%) were much improved by acetylation. The direct derivatization and extraction (v) also derivatized other material in the matrix which made the subsequent determination more difficult. Therefore, Brezney and Joyce [60] recommended that the chloroaromatics were extractively acetylated rather than directly.

3.4.4. Column extraction

When tissue or sediment contain low levels of contaminants, the sample mass has to be proportionally increased. Some workers [55] have homogenized large sample masses (500 g) prior to blending. However, bulk tissue is difficult to handle without large scale equipment. A simple alternative is to use a column extraction system. Smaller quantities of tissue can be ground with sodium sulphate and added to the glass column (1000 mm × 100 mm i.d.). Solvents such as dichloromethane are added and the tissue soaked for 30 min. The dichloromethane is slowly drained from the column and the extraction repeated. All extracted lipids and associated non-polar contaminants are removed with ca. 500 ml of solvent.

Allender [63] extracted chlorpyrifos from fish tissue in a single column. The glass column (200 mm × 9 mm i.d.) contained deactivated silica between anhydrous sodium sulphate and was washed with 1% acetic acid in *n*-hexane. The tissue (3 g) was ground with phosphate and sodium sulphate, tamped into the column and the chlorpyrifos eluted in diethyl ether in hexane (5:95). Recoveries of chlorpyrifos from the spiked fish were between 83 and 91% ($n = 3$).

3.5. COMPARATIVE EXTRACTION STUDIES

Ideally, the contaminants to be determined should be removed from the matrix as completely as possible with a minimum amount of the other non-target components. This

type of selectivity was certainly anticipated from supercritical fluid extraction following the successful production of decaffeinated coffee. However, trace organic contaminants cover a wide range of polarity, volatility and molecular size, making selective extraction very difficult to achieve. A comparison of the advantages and disadvantages of the main methods used to extract trace organic contaminants from sediment and biological tissue are given in Table 3.2.

Currently the most popular extraction methods are Soxhlet [43–47] blending [8,,50,52,53,55,56,57,60] liquid column extraction, ultrasonic extraction [64] and more recently supercritical fluid extraction [38,64–71].

The main comparisons between extraction methods have been made between the Soxhlet, sonication and supercritical fluid extraction [64,66,69,71]. This has primarily been prompted by the need to critically evaluate the relative merits of SFE as an alternative to the more established methods.

TABLE 3.2

COMPARISON OF THE MAIN METHODS OF EXTRACTION OF SEDIMENT AND BIOLOGICAL TISSUE FOR TRACE ORGANIC CONTAMINANTS

Method	Advantages	Disadvantages
Soxhlet	Multiple systems possible with banks of 6 or 12 Soxhlets; extraction is automatic once the system is set up; hot extraction to improve recovery	Relatively large volumes of solvent used; loss of some volatiles unless efficient condensers are used; solvent penetration limited if the sample is not completely wetted; thermally labile compounds may decompose; solvent and extraction thimble purity must be checked; thimbles require extraction before use
Supercritical fluid extraction	Relatively fast, depending on the temperature and pressure; uses non-toxic solvents; parallel extractions are possible; can be semi-automated; well controlled, reproducible conditions	SFE gas and modifier purity must be checked; high grade SF essential; limited size of extraction vessel; care on recovery from extraction vessel; losses can occur; blockages through the restrictors
Blender/ultrasonic	Simple, inexpensive to purchase and operate; applicable to a wide range of biological tissue and sediments; ambient temperature	Labour intensive; difficult to automate; may not extract contaminants bound to tissue or sediment; separation of extract and matrix debris necessary; filters may clog; limited size of sample
Column percolation	Large sample size can be extracted; cold extraction can be used in parallel; low equipment cost	Very large volumes of solvent used; high solvent blank; labour intensive

Richards and Campbell [64] made a comparison between SFE, Soxhlet and sonication methods for the determination of some priority pollutants in soil. The SFE apparatus was the same, relatively standard system as described by Campbell et al. [65] with the addition of a CO₂ cryogenic trap to improve the trapping of the more volatile extractants in dichloromethane. The priority pollutants selected were the five chlorobenzenes and six chlorophenols, *sym*-dichloroethyl ether and naphthalene. With a 2% methanol modifier in CO₂ at 390 atm and 80°C, the recoveries ranged from 70% for phenol to 83% for 2,4,6-trichlorophenol. The Soxhlet extraction used a 1:1 mixture of acetone and hexane for 16 h with recoveries ranging from 54% for 1,3-dichlorobenzene to 81% for 2,4-dichlorophenol. The sonication method used 1:1 acetone and dichloromethane and had recoveries in the range of 46% for 1,3-dichlorobenzene to 75% for hexachlorobenzene. They concluded that the SF was more efficient in extracting these compounds in less time. However, the following observations can also be made from these experiments and the data they presented.

- SFE recovery of phenol was 70%, but the phenol-*d*₆ was 95%. The 15% difference between the phenol and the surrogate, deuterated standard probably reflects the precision of the replicates. There were no data on the repeatability of the methods.
- Sonication of volatiles such as chlorobenzenes will result in losses, not necessarily from poor extraction, but because, with warming, they are lost to the atmosphere. A trap was put on the SFE apparatus, but not over the sonication equipment.
- Recoveries by SFE from a certified quality control sample were, with one exception, less than the certified value by a range of 15–50%.

Onuska and Terry [66] examined the extraction of TCDD from sediments. They found that either CO₂ or N₂O with 2% methanol as modifiers gave the highest recovery for TCDD at 310 atm and 40°C. They also studied the effect of extracting wet sediment, as opposed to the dry material, and found that when the sediment was moist, the recovery diminished by 20% for the same extraction time. However, the same efficiency could be achieved with the wet sediment if the extraction time of ca. 40 min was doubled. The Soxhlet extraction of the same dried sediment with *n*-hexane/acetone (1:1) (150 ml) and 2,2,4-trimethyl pentane (25 ml) for 18 h was only around 65% of the SFE recovery. The Soxhlet extraction was considerably more variable (22–90%, *n* = 3) but since the Soxhlet actually recovered 90% of the TCDD, this means that the method can be efficient but was erratic. This variability was almost certainly a function of the heterogeneity of the matrix surface and/or the wettability of the sediment. The BCR working group found that it was essential to wet the dried sewage sludge with electrolyte (5% ammonium chloride) for ca. 5 h prior to extraction and analysis of CBs [49].

Nam et al. [38] made a comparative study between SFE and liquid-liquid extraction (LLE) of eleven organochlorine pesticide residues and chlorobiphenyls, as Aroclor 1260, in blood and milk. The biological fluids were spiked at levels between 1 and 20 µg kg⁻¹, adsorbed onto florisil and extracted with CO₂. An aliquot of the same sample was solvent extracted with hexane (whole blood) or cyclohexane/dichloromethane (1:1) (milk). The recovery of OCs from the hexane extraction of the blood ranged between 77% and 107% and for the SFE from 72% to 91%. The recovery from the spiked milk, for the LLE, ranged between 72% and 110% and for the SFE it was between 77% and 91%. Although the recoveries were lower for the SFE, the purpose of this study was to attempt to obtain the maximum recovery without the need for further clean-up.

A comparison was made between the in situ analysis using SSJ/LIF and hot Soxhlet extraction for the determination of PAHs in sediments [13]. The study highlighted two aspects. Firstly, there was good agreement between the measurements made for both benz[a]pyrene and pyrene in the marine sediment by the two techniques (Table 3.3). The sediment used was a reference material prepared by the National Research Council of Canada (HS3). However, the second of the two soils sampled from the coal gasification plant did not produce the same level of agreement. There was considerably less PAHs found in these soils using the hot Soxhlet extraction with dichloromethane for 24 h at ca. 90°C (Table 3.3). Both Renkes et al. [72] and Junk and Richards [73] found inconsistencies in the recovery of PAHs when prolonged extraction times were used at elevated temperatures. This comparison clearly indicates the need to fully optimize the extraction conditions. Extended extraction time and extreme temperatures do not necessarily improve recovery and losses can occur through degradation.

Schuplan et al. [74] have used the "Bleidner" vapour phase extraction technique [75] for the determination of organochlorine pesticides and chlorobiphenyls in lake sediment and compared the results with traditional Soxhlet extraction. The advantage of the "Bleidner" distillation is that it avoids the time-consuming steps of drying, conventional extraction and clean-up. The thawed sediment was mixed with distilled water and an anti-foaming agent, and the aqueous phase distilled into a flask containing iso-octane, which was subsequently used to extract the distillate. Direct measurement of the OCPs and the CBs were made by capillary GC-ECD. The Soxhlet method required the sediment to be dried. The pore water was separated from the solid and extracted with *n*-hexane/toluene (9:1). The moist sediment was exhaustively dried with phosphorous pentoxide prior to Soxhlet extraction with *n*-hexane/toluene (9:1) for 20 h.

The recoveries for the CBs by the "Bleidner" technique declined with increasing chlorination (CB28 98% to CB180 43%) and was likely to be a function of the decrease in volatility of the congeners. The low recovery of γ -HCH (43%) was a result of the higher water solubility of this contaminant and the 4,4'-DDT (10%) and 4,4'-DDD (42%) was reported as a rapid metabolism to DDD and to dichlorobenzophenone, respectively. However, it is difficult to see why this does not occur with the Soxhlet extraction as well. The low recoveries of the DDTs by the "Bleidner" extraction is more probably due to the

TABLE 3.3

COMPARISON OF SUPERSONIC JET-LASER INDUCED FLUORESCENCE SPECTROSCOPY (SSJ/LIF) AND HOT SOXHLET EXTRACTION

Sample	Benzo[a]pyrene		Pyrene	
	SSJ/LIF	Soxhlet/HPLC	SSJ/LIF	Soxhlet/HPLC
Marine sediment		7.6 \pm 0.9 7.4 \pm 3.6	33.7 \pm 1.5	39 \pm 9
Soil from a coal gasification plant		180 102	700	371
143		24 245	53	

Values in mg kg⁻¹. Data in this table taken from Lai et al. [13].

stronger binding of these compounds to the sediment which is not reversed by simple stream distillation. The method, therefore, although rapid for some volatile, non-bound hydrophobic organics is not suitable for wide application as an extraction technique.

3.5.1. Micro-extraction methods

The mass of sample taken for analysis is primarily dependent on four factors: (i) the amount of material available; (ii) the concentration of the determinand; (iii) the heterogeneity of the sample; (iv) the method of analysis. Most conventional solvent extraction techniques currently use more sample than is required, use more extraction solvent than is necessary and ultimately only analyse 1/1000 of the material prepared, e.g. 1 μ l from 1 ml.

Micro-extraction techniques [76] can be used in conjunction with "on-line" LC-GC or LC-MS to utilize the whole extract in the final determinations. This approach can significantly reduce the size of sample required and the volume of solvent used. Steinwandter [76] has applied this technique to pesticide residues analysis of fruit and vegetables. A small mass, e.g. 5 g of fruit, water content >70%, is macerated and extracted with acetone or acetonitrile. A third non-polar solvent, petroleum ether or dichloromethane is added to the binary system which is then subsampled in situ for further analysis.

This extraction can be represented by the Gibbs triangle (Fig. 3.2). The whole procedure can be completed in a single vessel, or by isolating the original matrix by filtration prior to extraction into a non-polar solvent.

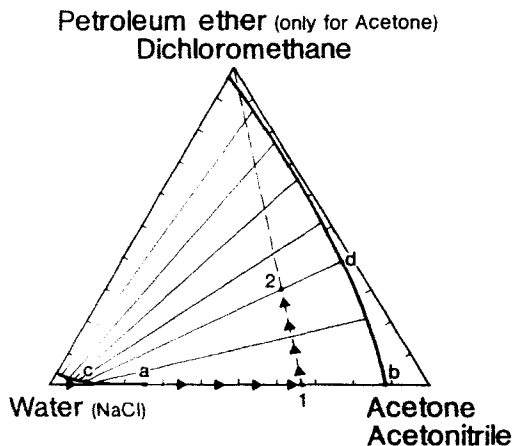


Fig. 2. Gibbs triangle representing the principles of micro extraction. After maceration and extraction with acetone and salting out with NaCl, a partially miscible solvent system, a and b, is extracted with a non-polar solvent. The two resultant solutions have compositions c and d. The organic phase, d, is drawn off for analysis [76].

3.6. CLEAN-UP

Normally, an extraction technique will be selected to give the highest recovery for a wide range of different contaminants. Therefore the extract will most likely contain a high proportion of co-extracted material. Many of the clean-up techniques have been tailored into a series of multi-residue schemes [8,46,55,57,77–80] in order to maximize the use of each sample. This is of particular value when the maximum amount of chemical information is required for each sample.

The main requirement for any clean-up and group separation scheme is that it effectively removes not only the bulk of the co-extractants, such as lipids, sulphur, carotenoids and pigments, but also those compounds that may potentially interfere in the final determination.

There are three main ways in which co-extracted material may interfere in the final determination, if not removed.

- (1) Gross contamination can overload the HPLC or GC columns with an obvious and usually rapid deterioration of chromatographic performance. This can occur with so-called “rapid” techniques where the detector is used as a filter, e.g. selected ion monitoring mass spectrometry, or where the clean-up method has been overloaded, e.g. as excess of lipid. This problem can be overcome by using and monitoring more selective clean-up techniques.
- (2) Interferences caused by inadequate chromatographic separation during the final determination, e.g. no prior group separation of CBs and OCPs or PCNs and planar CBs, OCPs and polychlorinated camphenes. This can be improved by multi-dimensional GC or multi-dimensional preparative LC.
- (3) The third type of interference occurs when compounds co-elute with the determinands and are not detected directly by a specific detector. The effect is to create negative peaks or an erratic response of the determinand. This problem can be identified by using a non-specific detector such as an ion trap detector, MS in EI mode or a flame ionization detector.

These problems are overcome by applying a tailored LC separation prior to the final determination and having a built-in feedback to monitor the success of the separation or to give a warning of any failure.

3.6.1. Measurement of extractable lipid

Both sediment and tissue extract are normally examined to determine the extractable organic residue. For sediments and soils, it is possible to compare the levels obtained in the organic extract with the total organic carbon determined by combustion techniques to verify the efficiency of the extraction. The standard method for the determination of extractable tissue lipids [81] forms a miscible mixture by blending with chloroform and methanol which is subsequently diluted with water and chloroform to form two layers. The chloroform layer is removed and evaporated to dryness in a tarred dish and the lipid content gravimetrically determined.

The lipids from other extraction methods are also measured gravimetrically. Some workers [47,77] take an aliquot of the extract to determine the extractable lipids while

others [8] evaporate the whole extract and re-dissolve the oil after weighing for the remaining analysis. The advantage of the latter method is that (i) it is likely to be more precise for low levels of lipid, (ii) none of the sample is lost, (iii) the solvent can be changed and (iv) the lipid determination is made on the actual fraction that is analysed. The disadvantage of the method is that volatiles such as chlorobenzenes can be lost during evaporation to "dryness".

The value of the extractable lipid measurement is twofold. Firstly, it indicates how much lipid has to be removed in the subsequent clean-up process and secondly, it allows the level of organic contaminants in the tissue to be expressed on a lipid basis. This normalization reduces the difference between different tissue purely as a result of the lipid in the tissue and the effect of external factors that affect lipid levels, such as seasonality.

3.6.2. Removal of lipids

There are four main methods used which will remove co-extracted lipids: (i) saponification; (ii) concentrated sulphuric acid; (iii) gel permeation; (iv) adsorption chromatography (Al_2O_3 , SiO_2 , and florisil).

3.6.3. Saponification

The bulk of the triglycerides and wax esters can be removed by the saponification of the extract with 20% potassium hydroxide in ethanol. However, this relatively harsh treatment is unsuitable except for the most chemically resistant contaminants. Most organophosphorus pesticides are hydrolysed by this method. The 2,4'- and 4,4'-DDT are dehydrochlorinated to the corresponding 2,4'- and 4,4'-DDE and the hexachlorocyclohexanes are also degraded. Saponification has been used successfully for the determination of some CBs [48,49], but the more highly chlorinated CBs are prone to loss of chlorine especially if the reaction is undertaken at too high a temperature, e.g. $>70^\circ\text{C}$ for too long a period of time, $>1\text{ h}$ [82,83]. It is therefore essential to check the recovery of each compound to be determined under the specific conditions of the reaction if this technique is to be used. Although this may be a disadvantage for the higher chlorinated CBs, it is possible to make use of these hydrolysis reactions as confirmation of the presence of more reactive compounds, e.g. HCH, DDTs and esters.

3.6.4. Sulphuric acid

The other main harsh chemical method used to remove the bulk of the co-extractants is the dehydration and oxidation reactions with concentrated sulphuric acid. Again, this method is only suitable for the most robust chemical groups such as organohalogens without an oxygen bridge, i.e. not suitable for dieldrin, endrin and aldrin [8,47,52,84]. The initial methods involved shaking the determinand in an alkane solvent with the concentrated acid. However, this reaction is more manageable if the acid is adsorbed onto silica gel. Up to 40% of sulphuric acid w/w can be loaded onto the silica. The value of this method is that up to 20 g of lipid can be effectively denatured by passing the sample through a column containing 50 g of the 40% H_2SO_4 on SiO_2 and eluting with dichloro-

methane [47,84]. It is possible to automate the clean-up in a batch process in a gravity column or a low pressure flow-through system. The columns or cartridge are used once and discarded.

3.6.5. Solid phase clean-up

Liquid chromatographic clean-up has been used either in normal phase using alumina, silica or florisil [8,45,77,84,85] or with reverse-phase (RP) columns [50,56,80] (Table 3.4). In most cases, these techniques are well established and are used in an "off-line" mode, primarily to remove the bulk of co-extracted materials prior to a more refined clean-up prior to the final determination. These columns may be prepared in the laboratory [43,45,77,85] or use can be made of solid phase extraction (SPE) cartridges [50,56,68,80,86,87]. In both cases, the normal phase cartridges and column materials are disposable since many of the polar co-extractants bind firmly to the surface and are difficult to remove. This has been overcome to some extent using RP materials where the polar compounds are eluted prior to the non-polar materials. These columns and cartridges

TABLE 3.4

METHODS FOR THE CLEAN-UP OF TRACE ORGANIC CONTAMINANTS IN SEDIMENT AND BIOTA: ADSORPTION AND REACTION COLUMNS

Application	Solvent	Column	Reference
Toxaphene in biological tissue	<i>n</i> -Hexane/diethyl ether	Sulphuric acid	Jansson and Wideqvist [116]
PAHs in crude oil and sediments	<i>n</i> -Hexane	SPE florisil	Garrigues and Bellocq [87]
Pyrethroids in fish eggs	Acetonitrile/water/hexane	SPE C ₈ alumina/silica gel	Bolygó and Hadfield [56]
Polychlorinated diphenylethers in human adipose tissue	<i>n</i> -Hexane	Sulphuric acid, modified silica	Stanley et al. [54]
Tetra chlorobenzyl toluenes (Ugilec)	<i>n</i> -Hexane	Basic alumina, silica gel	Wester and Van der Valk [43]
PAHs in sewage sludge and soils	Dichloromethane	SPE florisil	Wild et al. [68]
PAHs in sediment	Methanol/dichloromethane (2:3)	SPE silica	Morel et al. [117]
Toxaphene in fish tissue	<i>n</i> -Hexane <i>n</i> -Hexane/diethyl ether (75:25)	Basic alumina, silica gel	Van der Valk and Wester [45]
Organochlorine pesticides in milk	Milk/methanol (1:1)	SPE C ₁₈	Redondo et al. [50]
Organochlorine pesticides in animal feed	Acetone/water (1:1)	SPE C ₁₈	Torreti et al. [80]

can be regenerated in some circumstances by flushing with methanol, but quite often the gross contamination from the co-extractants prevents them from being re-used. Similar LC clean-up and separations are used "on-line" for less contaminated samples (see later).

3.6.6. Gel permeation chromatography (GPC)

Most workers [8,44,46,55,57,88,89,90] use SX3 Biobeads (200-400 mesh) in a range of column sizes and solvents (Table 3.5). Separation has been made primarily between lipid material >500 Å which is the first to elute from the column followed by the smaller molecules which include most of the organic contaminants that accumulate in biological tissue. This separation is particularly appropriate for the determination of trace organic contaminants in fish since most of these compounds are bio-accumulated via the gill filaments, which have an upper molecular size cut-off of ca. 500 Da.

TABLE 3.5

METHOD FOR THE CLEAN-UP OF TRACE ORGANIC CONTAMINANTS IN SEDIMENT AND BIOTA: SIZE EXCLUSION CHROMATOGRAPHY

Application	Column	Eluant	Reference
PAHs, nitro PAHs and derivatives in smoked foods	340 mm × 25 mm Biobeads SX3	Dichloromethane/ cyclohexane (1:1)	Larsson et al. [55]
PAHs and chlorinated hydrocarbons in sediment and biota	100 Å SEC column	Methylene chloride	Krahn et al. [78]
Alkyl biphenyls, linear alkyl benzenes PCB replacements in sediment and fish	Biobeads SX3		Peterman and Delfeno [118]
Chlorobiphenyls in animals fat	600 mm × 25 mm Biobeads SX3	Ethyl acetate/ cyclohexane (1:1)	Tuinstra et al. [88]
Chlorobiphenyls in reindeer, fish and seal	50 mm × 10 mm Biobeads SX3	<i>n</i> -Hexane	Haglund et al. [89]
Chlorinated terphenyls Aroclor 5432 and 5460 in fish lipid	600 mm × 25 mm Biobeads SX3, SX8	Dichloromethane/ cyclohexane (1:1)	Hale et al. [44]
43 Organophosphorus pesticides in plant and animal tissue	300 mm × 25 mm Biobeads SX3 60:40	Hexane/ethyl acetate	Holstege et al. [57]
Chloro and bromo hydrocarbons in biota	300 mm × 25 mm	Dichloromethane/ hexane (1:1)	Jansson et al. [8]
OCPs and OPs in fish tissue	450 mm × 10 mm Biobeads SX3	Ethyl acetate/ cyclohexane (1:1)	Porte et al. [46]

GPC or size exclusion chromatography (SEC) has several key advantages over all other methods currently available. The method is non-destructive and, unlike saponification or concentrated sulphuric acid clean-up, can be used to isolate less robust contaminants, e.g. organophosphorus pesticides [46,57]. It is also more applicable to the isolation of "unknown" contaminants where there is little information on the polarity or chemical functionality of the molecule. Adsorption chromatography is not able to isolate groups of compounds with very different polarities or structure in a single small fraction. GPC is also considerably more tolerant of handling a large mass of lipid in each sample. Columns of ca. 500 mm \times 25 mm i.d. can cope with up to 500 mg of fat whereas the adsorption columns are limited to ca. 50 mg g⁻¹ of lipid. It is possible to increase the size of the adsorption column to remove ca. 250 mg of lipid, but larger volumes of solvent are required to elute the more polar organics.

One main disadvantage of the GPC system is that it is difficult to completely remove all traces of the lipid [91]. Since the triglycerides elute prior to the smaller contaminants, the "tail" of the lipid peak intrude into the second fraction. The amount of lipid in the "tail" becomes significant where a relatively large mass of triglyceride has to be removed relative to the concentration of the contaminants. Grob and Kälén [91] found that much of the tailing was caused by lipid trapped in the injection port and the connecting tubing. Although this contamination was reduced by appropriate switching, the lipid was not completely eliminated. Even a 0.01% carryover from 1 g of lipid will leave an unacceptably high level of co-extractant in the extract. Until this inherent problem can be solved, the low molecular weight fraction usually requires further clean-up to remove the trace lipids, e.g. SiO₂ prior to analysis.

Jansson et al. [8] were able to use the SX3 Biobeads and a mobile phase of dichloromethane in hexane (1:1) to make a further separation of the chloroparaffins from the lipids and other organochlorine contaminants. Using diethylhexyl phthalate (DEHP) as a marker, they collected the fraction 1.15–1.44 times its retention time to isolate the chloroparaffins and the second fraction 1.44–2.46 times the retention of DEHP for the other contaminants.

3.6.7. Supercritical fluid clean up

Most SFE studies have focused on obtaining a complete separation between the bulk matrix and the small organic contaminants ca. <500 Da in situ. With a few exceptions [38,41], the extraction removes some or all of the soluble lipophilic material along with the trace organic contaminants. The difficulties in selecting the optimum SFE parameters to obtain a lipid free sample has been a limitation of the SFE method and of the hyphenated SFE-SFC. France et al. [92] have proposed a method for the supercritical fluid clean-up of fatty tissue extracts by injecting the fat extract on to a column 70 mm \times 4.6 mm i.d. containing alumina (activity Brockman I) and eluting with CO₂ into *n*-hexane. The column, once used, was discarded. Silica was also used as a clean-up adsorbent in the same column with the advantage that it could be re-used, once all of the extract was removed. After the elution of the pesticides, the lipids, which were trapped on the silica, were eluted with 5% methanol/CO₂ in a step gradient elution. The lipid fraction was discarded through the back pressure regulator to prevent the restrictor from becoming blocked.

Lohleit and Bächmann [70] used adsorbents such as Tenax, Carboxpack C, Spherosil XOA200, florisil and reverse phase C₁₈ sorbent to trap organics and subsequently desorb them using SFE with CO₂. These adsorbents can be used to trap the contaminants from air, but also from SFE extraction. Carboxpack was unstable when used with supercritical fluids and high molecular weight artefacts were extracted from Tenax.

3.6.8. Sulphur removal

Elemental sulphur is present in most soils and sediments, and is sufficiently soluble in most common organic solvents that the extract must be treated to remove the element prior to analysis by GC-ECD or GC-MS. The most effective methods available are: (i) reaction with mercury or a mercury amalgam [74] to form mercury sulphide; (ii) reaction with copper to form copper sulphide; or (iii) reaction with sodium sulphite in tetra-butyl ammonium hydroxide (Jensen's reagent) [93]. Removal of the sulphur with the mercury or copper requires the metal surface to be clean and reactive. For small amounts of sulphur, it is possible to include the metal in a clean-up column. However, if the metal surface becomes covered with the metal sulphite, the reaction will cease and will need to be cleaned with dilute nitric acid. For larger amounts of sulphur, it is more effective to shake the extract with Jensen's reagent [93].

3.7. AUTOMATION

Automation per se does not always remove the problems of time and effort associated with manual methods. A critical evaluation of both the manual methods to be replaced and the automated alternative should be made before embarking on a new scheme. "New, improved and rapid" methods in the literature may not always be appropriate. For example, a bank of 12 Soxhlets, with safety cutouts, can be left unattended overnight and is less labour intensive than SFE which, although only takes 10–12 min per extraction, requires manual loading for each sample. Soxhlet extraction has been estimated to be less expensive per sample [38]. HPLC clean-up using silica or alumina may be no quicker than the equivalent LC gravity columns which can operate in parallel (12–18 samples) also overnight at considerably less cost.

3.7.1. Robotics

Simple manipulations in sample preparation remain one of the most labour intensive areas of analytical work, almost regardless of the methods of pretreatment [94]. There are many applications of auto-injection, multi-dimensional chromatographic separations and data analysis, but sample preparation has not had the same level of automation in most laboratories. The key advantages of automation are:

- unattended repetitive tasks (time saving);
- greater accuracy, consistency, reliability and less prone to fatigue than manual methods;
- continuous operation with toxic solvents (dichloromethane) and corrosive materials

(SiO₂/sulphuric acid, also fine powder adsorbents) (safety)

- automated systems may require isolation, but not fume cupboards (saving space and cost).

Robotic systems in a small analytical laboratory have the greatest application in the intermediate sample manipulation steps. The removal of excess solvent with the Zymark evaporator, for example, can be very closely controlled, fully automated and operate in parallel (up to six samples per instrument). This technique has considerable advantages over rotary evaporation, which is prone to lose volatile organics, e.g. chlorobenzenes and HCH etc. under vacuum and rapid vaporization. More conventional blowdown apparatus requires considerable fume cupboard space. Automated repetitive manipulations are well served by a robotic system [95]. Small volumes of volatile samples are best controlled by weight and aliquots can be taken automatically and the weights recorded electronically.

3.7.2. On-line automation

Although automation and more especially “on-line” systems offer considerable attractions, such techniques need to be fully investigated before applying them to an analytical manipulation. The relative advantages and disadvantages of the “on-line” approach with the present state-of-the-art are given in Table 3.6.

The transition from a manual to an automatic method is more easily made if each step in the existing method is readily amenable to such a change, e.g. column extraction or SFE are good candidates for automation, but blending would only be suitable with extensive robotics. Most LC methods, whether gravity columns, SPE or HPLC can be automated and connected “on-line” to the final GC stage. However, there are two main unresolved problems with the “on-line” LC-GC approach for multi-residue analysis.

Although the separation between some unwanted co-extractants and the determinands is well suited to an “on-line” system, high fat loading or elemental sulphur are more effectively removed “off-line”. Most “on-line” systems at present work most effectively with low lipid content, e.g. drinking water [96–98] although some applications have, in part, overcome the problem of lipid removal [99].

The LC-GC is used to isolate the determinands in a separate fraction from other interferences, usually by heart-cutting, and then to chromatograph that fraction by GC. However, difficulties arise when multiple fractions must be isolated from each sample by the LC, for example different groups of compounds, e.g. CBs and OCPs. The sample also needs to be separated into fractions when similar compounds are present at considerably different concentrations or where chromatographic overlap is to be avoided. Under such conditions, the multiple fractions produced by the “on-line” LC cannot be analysed directly by linking a single GC.

This difficulty may, however, be overcome by one of the following configurations:

- by an “on-line” heart cut into the GC autosampler, so that each fraction can be taken sequentially into the GC. There are two disadvantages of this approach. Firstly the GC column phases may need to be different for each fraction to obtain the appropriate separation and secondly the inherent sensitivity of the “on-line” system is lost.
- by an “on-line” LC heart cut into separate parallel GCs. This can be a practical option for laboratories that use multiple GCs which are optimized for the analysis of

TABLE 3.6

ADVANTAGES AND DISADVANTAGES OF ON-LINE LC-GC WITH THE PRESENT STATE-OF-THE-ART TECHNOLOGY

Advantages	Disadvantages
Rapid, direct analysis with considerable saving in time	Potential loss of high resolution in coupling; concurrent solvent evaporation needs very careful matching of conditions to have the same precise focusing as on-column injection of small volumes
Use of all the sample with considerable increase in sensitivity where appropriate or reduction in the initial sample volume	Limited to on-column injection techniques with a minimum of 80°C difference in temperature between solvent and the first determinand
Reduction in the amount of solvent used, especially with narrow or microbore LC	Not possible to analyse multiple eluates from the LC; multi-residue analysis is difficult
Less chance of introducing an artifacts in the sample	Separation of interfering, co-eluting compounds not always possible
With less solvent there are lower levels of impurities	Present systems cannot cope with high lipid loading (ca. >500 mg)
Avoids loss of solute	Desulphurization and large amounts of lipid to be removed "off-line"
On-line group separation and sample preconcentration is possible	Memory effect can cause interferences; lengthy optimization and set time
Provides two independent sets of retention data for correlation to improve identification	All the sample is used in a single shot analysis. No chance to repeat analysis or determine other compounds in the extract

each fraction in a multi-residue scheme.

- some form of stop-flow LC and sequential GC analysis.

Despite the difficulties of "on-line" automation, the need to develop such systems is considerable. The increase in the number of different compounds that must be determined and the number of samples required for a meaningful survey or laboratory study make it essential to improve the quality and throughput of samples.

There are a number of stages in fully automating trace organic analysis. Autosampler-LC or GC-Data Systems, hyphenated techniques GC-MS, GC-ITD ion trap detector are well established and require no further elaboration here. MDGC has been used to great effect in the separation of closely eluting compounds, cf. CBs [100,101] and PCDDs [102], but this technique is neither used for routine quantitative analysis nor available to a large number of workers at present.

The early developments of on-line LC-GC have been reviewed by Davies et al. [103] and Koenigbauer and Major [104]. The selectivity characteristics of the mobile phase and stationary phase can be optimized to give both a cleaned-up sample and group separation by heart-cutting the desired fraction prior to GC analysis.

The LC is usually interfaced to the GC via an uncoated, deactivated GC capillary precolumn to transfer the heart-cut from the LC. This heart-cut from the LC is vaporized to focus the solute at the head of the column [105]. The volume of the GC precolumn, the volume of the "heart-cut" and the GC temperatures and carrier gas flow for the concurrent solvent evaporation are carefully matched [106,107].

The following examples highlight the progress and the pitfalls of the art of "on-line" LC-GC applications in environmental trace contaminant analysis. Maris et al. [96] determined CBs in sediment by "on-line" narrow bore LC-GC. One key advantage of using the narrow bore columns is the inherent low LC flow rates of ca. $5\text{--}50\ \mu\text{l min}^{-1}$ which are more comparable to coupling to the GC. The $150\text{ mm} \times 1.1\text{ mm i.d.}$ column was packed with $5\ \mu\text{m}$ LiChrosorb Si60 and a $20\text{ mm} \times 0.7\text{ mm i.d.}$ LiChrosorb AloxT guard column. The sediment was extracted in a Soxhlet and the sulphur removed with Jensens' reagent [93]. Hexane was used as the LC solvent at $18\ \mu\text{l min}^{-1}$ and the CBs were heart-cut into the GC between 5 and 10 min after injection onto the LC. A comparison was made between the LC-GC and the "off-line" alumina-silica clean-up, and the data obtained for the two methods for the seven monitoring CBs 28, 52, 101, 118, 138, 153 and 180 were quite similar; however, the following observations highlight some difficulties that may occur with this method.

- The sediment was extracted in hexane. A more polar solvent, e.g. dichloroethane may extract more CBs, and almost certainly more co-extractants that would need to be removed by the LC.
- The LC alumina guard column would deteriorate quickly with multiple samples, and even with backflushing and would need to be replaced regularly.
- The GC used was a widebore (0.32 mm) column with N_2 as a carrier gas. Such a system has a considerably lower resolution, as evidenced by the chromatograms, than would normally be required of the high resolution separation with H_2 or He and a 0.22 mm i.d. column [49,101]. The lower performance of this widebore column may mask any band broadening at the LC-GC interface.
- The separation of the CBs from the pesticides and other OC residues was not possible with this "on-line" system and would seriously interfere (i) with the determination of the OPCs themselves and (ii) other CBs.

The technique clearly has the advantage of speed and improved sensitivity since the whole sample extract is used. After some development to improve the resolution of the final determination, it may be appropriate for the analysis of a small number specific CBs in a routine monitoring programme.

Barcarolo [99] used a more conventional bore LC to interface to the GC for the determination of OCPs in fat. Butter was melted and diluted to ca. 5% in iso-octane and the resultant solution injected directly into the LC. The LC column used was a $40\text{ mm} \times 4.6\text{ mm i.d.}$ BioSil OSD-10 C_{18} system with iso-octane as an eluant at $85\ \mu\text{l min}^{-1}$. The OCPs were heart-cut into the GC after which the fat was removed from the LC column with hexane at a flow rate of 2 ml min^{-1} and diverted to waste. The OPC fraction ($680\ \mu\text{l}$) was transferred to the GC using full concurrent evaporation over an 8-min period and chromatographed on a $50\text{ mm} \times 0.32\text{ mm i.d.}$ SE 52 column using H_2 as carrier. The reproducibility of this system was between 2 and 15%, $n = 10$, with a sample intake mass of ca. 2 mg per sample. The separation of the OCPs and the triglycerides was almost

100%. Degradation of the LC column by fat retention occurred after 20–30 samples, but the LC performance was restored by reverse-flow washing with hexane for 18–20 h (e.g. overnight). The column still functioned well after 200 or more samples. Some co-extracted material was evident from the chromatograms and this technique would benefit from having an ITD or MS in tandem to confirm the purity and identity of the eluting compounds.

René et al. [98] used SPE cartridges in an ASPEC (automatic sample preparation with extraction columns) system coupled with capillary GC-ECD to determine OCP and pyrethroid insecticides. Hexane (2 ml) extracts of drinking water or surface water were passed through the Bakerbond SPE cartridges containing 100 mg of silica. The OCPs were eluted with *n*-hexane/iso-propanol (99.9:0.1) and injected into the GC using a 6-m fused silica retention gap; the sample introduction time being matched to give concurrent solvent evaporation. The samples were effectively cleaned-up on the SPE and 17 OCPs were isolated from co-extracted material and the pyrethroids selected. Since the GC analysis time was 70 min, the sequential sample was prepared by the ASPEC in parallel to the GC determinations. The recovery of the 23 pesticides tested ranged from 95 to 107% with a CV% of between 7.5 and 11.8% and a limit of determination of between 3 and 30 ng l⁻¹.

Kapila et al. [41] used an “on-line” SFE-LC to determine chlorinated phenols in wood chips over the concentration range 1–500 mg kg⁻¹. Following the extraction, the sample was loaded into a sample loop of the HPLC and chromatographed using a conventional packed LC column and UV detector. Neilen et al. [108] coupled a SFE system with a GC-ECD for “on-line” determination of CBs which had been trapped onto solid absorbents such as Tenax. Their application was primarily to determine OCs in the atmosphere, but such a system could be adapted to trap a cleaned-up extract of a biological tissue prior to the analysis by GC-ECD or MS.

3.8. MULTI-RESIDUE SCHEMES

At present the serial “on-line” approach is difficult to fully incorporate into the multi-residue scheme [8,78] in which a large number of compounds are separated into groups and determined in parallel. The value of the multi-residue method permits:

- an extensive analysis of expensive and sometimes irreplaceable samples, especially those taken from remote sites, e.g. open ocean or from specific experiments;
- correlation of data of different determinands within a single analysis to reduce variability;
- the reduction of analytical effort at the sample preparation stages.

Multi-residue schemes are used by a number of workers for the determination of very different compounds [8,46,49,55,57,77,79,80] and each of the methods of extraction and clean-up discussed in the earlier part of the chapter have been incorporated into an overall analytical scheme.

Jansson et al. [8] used the conventional approach of blending the soil or biological tissue with solvent after which an aliquot was taken to determine the volatile compounds, e.g. phenols and chlorobenzenes. A second fraction was taken after the lipid determination for compounds sensitive to concentrated sulphuric acid, e.g. the drins. The bulk of

lipids were removed by oxidative dehydration with $\text{SiO}_2/\text{H}_2\text{SO}_4$ and further cleaned-up with GPC. The chloroparaffins were isolated at this stage. Separation on silica isolated the OCPs and the organochlorines and organobromines were finally fractioned on active charcoal.

Krahn et al. [78] have developed a similar multi-residue scheme for the determination of OCs and PAHs in sediment and biota. In this scheme, the preparation is semi-automated with GPC to separate the biogenic material from both the PAHs and the OCs and the sulphur in the sediment samples. The faecal sterols were separated and cleaned up with an amino-cyano HPLC column prior to derivatization with bis(trimethylsilyl) tri-fluoroacetamide. The previous, manual scheme used alumina and silica clean-up columns, which took considerably more time.

Multi-residue schemes have been reported for the samples of fruit and vegetables [5], carbamate pesticides [109], organophosphorus pesticides [6], organo nitrogen pesticides [7] and headspace analysis for dithiocarbamates [110].

3.9. DIRECT METHODS

In some situations, it is possible to undertake the measurement of some trace organics by direct measurement, thus avoiding the concentration and separation steps. Johnston and Bauman [111] used HPCL-fluorescence spectroscopy to determine the environmental exposures of fish to benzo[a]pyrene. The bile of the fish were collected in a light proof vial and the contents injected directly into a 250 mm \times 2.6 mm i.d. ODS HCSil X-1 Vydak HPLC column. Acetic acid/water/methanol were used in the gradient elution and the benzo[a]pyrene measured at 380–430 nm excitation emission. Other PAHs and PAHs metabolites can also be measured using a similar technique [78].

Deshpande [112] used a modification of Krahn's procedure for the separation of fish biliary PAHs metabolites using three C_{18} columns in tandem. The fish bile was injected in water as the starting eluant and the gradient elution changed to 100% acetonitrile over 240 min. The slow elution allowed the system to be flushed free of precipitable material while maintaining the necessary separation. A total of 21 PAHs metabolites and conjugates were separated and measured by this method.

Ariese et al. [113] applied the low temperature Shpol'skii spectrofluorometric technique to the direct measurement of PAHs in tern and mussel tissue, but the sensitivity of the determination was affected by the presence of lipophilic material in the crude extract resulting in a poor quality Shpol'skii matrix. This powerful technique, which offers an alternative to conventional GC detection, requires similar pretreatment to remove the co-extracted material in order to obtain a strong spectrum and improved detection.

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Chapter 4

Current developments in the analysis of polychlorinated biphenyls (PCBs) including planar and other toxic metabolites in environmental matrices

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4.1. INTRODUCTION

Since Jensen's initial detection of polychlorinated biphenyls (PCBs) in biological tissue in the 1970s [1,2] and the subsequent realization that these toxic compounds were potentially harmful both to man and to wildlife, there has been a continuous development in

both the analytical techniques to determine these chemicals [3–7] and in the assessment of their biological effect [8,9]. PCBs have been manufactured in substantial amounts since the 1920s [10]. Their use in the electrical, paint, pigments, paper and cardboard industries and subsequent disposal into the environment during the intervening years allowed sufficient time for them to spread to the remotest areas of the world before any control on use or disposal was implemented. Their high hydrophobicity, lipid solubility and persistence has resulted in widespread contamination of biological tissue to the extent that all environmental compartments that have been analysed contain measurable levels of these contaminants [11–15].

The early measurements of the PCBs were made using packed gas chromatographic columns and industrial formulations to quantify a total value for the PCB [4]. This early technology did not have the resolution to separate individual chlorobiphenyls (CBs) and the most appropriate method to estimate these contaminants at that time was unquestionably by the summation of the peak heights or areas of the low resolution chromatogram. Some workers recognized the potential errors in such estimates and attempted to obtain a single response by perchlorination to the decachlorobiphenyl (CB 209) [4,19,20]. The need to improve the separation, identification and quantification of the individual CBs has been reinforced by measurement of the toxic, biological effects of specific congeners [8,9,21,22,23]. The key developments in the measurement of CBs since 1966 are summarized in Table 4.1. With the present technology and available methodology [24–26], it is now possible to measure individual CBs routinely at the pg kg^{-1} , and with care at the fg kg^{-1} .

This chapter reviews the recent developments in the determination of individual CBs, including the separation of the non-*ortho* chloro- and mono-*ortho* chlorobiphenyls, and their metabolites in environmental samples which have been reported, primarily, since 1988. The information is presented in the same sequence that an analyst would approach the work; beginning with the selection and the final measurement of the determinands, followed by the preparation and separation stages and the initial extraction procedures.

4.2. SOURCES OF ERROR

The technology for the accurate determination of individual CBs in environmental matrices is available and many of the problems associated with these measurements are now reasonably well understood and documented [27–30]. A summary of the key problems encountered in these analyses, the symptoms and the remedies are summarized in Table 4.2.

4.3. COMPOUND SELECTION

There are 209 possible CBs of which 132 have been determined in industrial formulations at or above the 0.05% level [5]. Most analysts agree that it is currently unnecessary, and certainly undesirable to determine all CBs in every matrix. The selection of CBs will depend on the purpose of the measurement, which may differ from programme to programme and the ability of the laboratory to make that measurement.

TABLE 4.1

CHRONOLOGICAL DEVELOPMENT OF CHLOROBIPHENYL ANALYSIS

Year	Development
1925	Large scale manufacture of PCB formulations
1966	First reported measurement of PCBs as "Avian Peaks"; packed GC column separation of PCBs; concentration in samples estimated against industrial formulations and summation of mixed component peaks in the chromatogram
1969	Development of adsorption column chromatography for clean-up of biological tissue
1975	Introduction of glass capillary columns. Improved separation of PCBs
1980	Individual chlorobiphenyl congeners identified and systematically numbered; introduction of fused silica capillary columns. Improved column stability
1983	Development of stable GC ovens and electronics to improve reproducibility of retention indices
1984	Retention times of all 209 CBs measured on a SE 54 capillary column
1985	Reference materials, certified for individual CBs become available; commercial availability of many of the 209 CBs; reports of retention times on polar and semi-polar stationary phases; development of multi-dimensional chromatography
1988	Focus on the analysis of planar, toxic CBs and the application of toxic equivalence concentrations (TECs) for CBs as well as dioxins
1989	Separation of CBs on the basis of their spatial configuration. Identification of all congeners present in main commercial formulations
1992	Expansion of retention data for 5 GC phases of different polarity for all congeners in commercial mixtures (>0.05%), except CB 69, 75, 96 and 182

In the past, the selection of CBs for analysis has been largely based on persistence, relative abundance, toxicity, and the unequivocal measurement of the CB in a specific matrix. The initial selection of seven CBs made by the Community Bureau of Reference in 1982 was based on these criteria and the analytical technology at that time [31] (Fig. 4.1). For many workers, this selection was a valuable starting position for general monitoring work, but is clearly insufficient when relating the measurement of specific CBs to toxicological effects. Recent analytical developments have centred on the determination on the non-*ortho* chloro- and mono-*ortho* chlorobiphenyls [8,9] which are known to be toxic and induce liver microsomal enzyme activity. These CBs induce both aryl hydrocarbon hydrolase (AHH) and ethoxy resorufin-*O*-deethylase (EROD), bind with high affinity to the cytosolic receptor protein and are iso-electronic with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Both the toxic mechanism and the enzyme induction involve an initial binding of the CB to the same arylhydrocarbon, Ah, receptor.

Studies on the measurement of the toxic, planar CBs has been given added impetus by the correlation of EROD measurements with the determination of the toxic equivalent concentrations (TEC) [8,9,13,32]. One consequence of this emphasis in the determination of CBs is that a number of studies have focused on the one group of congeners alone [33–38], sometimes to the apparent exclusion of the other CBs.

Toxic effects, other than that of AHH/EROD induction, are well known [9,39], in particular phenobarbital (PB) induction, and dopamine reduction. Seegal et al. [40] found that *ortho*-substituted CBs which did not assume a planar configuration reduced the dopamine content in non-human primate brain cells, decreasing psychomotor and cogni-

TABLE 4.2

SUMMARY OF THE MAIN SOURCES OF ERROR IN THE DETERMINATION OF CBS IN ENVIRONMENTAL MATRICES

Problem	Symptom	Remedy
Calibration	Accuracy is concentration dependent	Use multi-point calibration to mirror detector response; check optimum make up gas flow for maximum "linearity" not maximum sensitivity
Inaccurate calibration solution	High/low bias in intercalibrations	Prepare independent calibration solutions and double check with a second laboratory
Calibration solution evaporation	Calibration response increases with time	Control calibration solutions by weight; use new, ampouled solutions
Impure calibrants	Multiple peaks in single calibration solution	Use certified calibrants
	Bias from low calibrant response	Check all calibration solutions by FID as well as EDC.
Internal standards	All determinands have lower or higher values than expected	Incorrect addition of internal standard; only use compounds that do not occur in samples
Poor GC resolution	Shoulders, unresolved doublets or single peaks; results biased high	Use 50 m narrow bore (0.22 mm i.d.) columns; check peak purity with a second/third column, MS and/or MDGC; optimize carrier gas flow rate; only use He or H ₂
High mass discrimination	Tail-off of sensitivity with retention time	Clean splitless injector; optimize split temperature/ time; use on-column injection
Co-extracted material	Noisy baseline Negative peaks	Improve clean-up Sulphuric acid and/or aminopropyl HPLC clean-up
	Interference from other OCs	Use RP-HPLC and PGC or pyrenyl HPLC to remove PCNs, PCDD/Fs and CPs
Poor recovery	Low or varied results	Check and validate method; use surrogate recovery standards CB 53, CB 189, ¹³ C labelled CBs

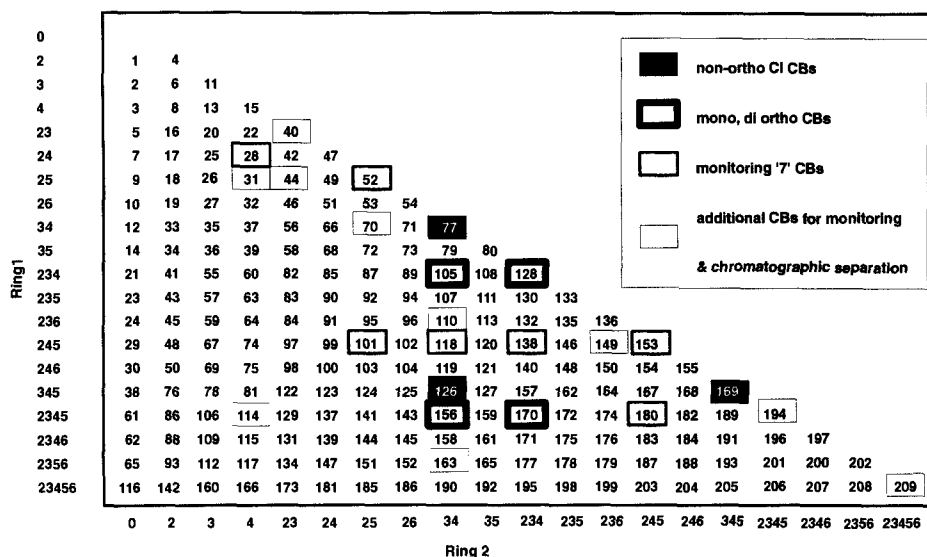


Fig. 4.1. Systematic numbering of the 209 chlorobiphenyls, according to Ballschmiter [125] giving the corresponding substitution patterns on each of the two biphenyl rings listed on the axis of the table. The outlined boxes indicate those CBs which are more commonly determined.

tive function. These were CB 4, 2,2'-dichlorobiphenyl, CB 17, 2,4,4'-trichlorobiphenyl, CB 47, 2,4,2',4'-tetrachlorobiphenyl, CB 50, 2,4,6,2'-tetrachlorobiphenyl, and CB 52, 2,5,2',5'-tetrachlorobiphenyl. The planar congeners CB 77, 3,4,3',4'-tetrachlorobiphenyl and CB 126 3,4,5,3',4'-pentachlorobiphenyl had no effect on dopamine levels. They found that chlorination in the *p* position of at least one ring increased the congener potency, but that substitution in the *m* position had the reverse effect.

Paivi et al. [41] studied the induction of AHH activity in mice and rats by two previously uncharacterized CBs. One was the non-*ortho* substituted 3,4,5,3',5'-biphenyl (CB 127) and the other was the mono-*ortho* substituted 2,3,4,3',5'-pentachlorobiphenyl (CB 108). Neither of these CBs have, to date, been included in any environmental analytical programme and both compounds are commercially available as individual congeners.

These examples illustrate the dynamic nature of our present understanding of the analytical requirements and the selection of congeners in relation to biological effect. While the selection of CBs depends very largely on the programme objectives, it is generally advisable not to restrict the measurement to a few specific toxic congeners when so many competing biological reactions have been identified. Equally the choice should not be limited to the abundant, less reactive CBs, e.g. CB 153/CB 138/CB 180 since information on biologically active congeners is more urgently required. Extrapolation of data on the more abundant CBs to estimate levels of the ultra trace, toxic CBs is not possible since the ratio of congeners between species, between different tissues of the same species and even between replicate samples are far too varied.

4.4. DETECTION

4.4.1. Calibrants and calibration

The earlier measurements of PCBs were dependent on industrial formulations to calibrate the detector and to quantify the total PCB concentration in the sample [4]. This calibration was usually achieved by peak matching and summing selected peaks to produce a total value. Considerable effort has gone into the production of individual, pure chlorobiphenyls, initially by individual research laboratories and now by commercial companies and reference material suppliers. Selected CBs are available as certified or well characterized materials for monitoring and for toxicological studies. Of the 209 CBs, 124 are currently commercially available and these cover more than 85% of the CBs found in the environment. The main CBs which are not widely available have the substitution patterns 2,3,5 (2), 2,3,6 (6), 2,6 (8), 3,5 (12), 2,3,4,6 (9) and 2,3,5,6 (9). The values in parentheses are the number of each substitution pattern currently not available.

The calibration of the GC using PCB formulations is a legacy from the earlier work and should now be regarded as inappropriate laboratory practise [42,43]. It is difficult to attain the necessary level of accuracy and precision required [29] and to probe the present problems relating the environmental processes and toxicological studies [27] with these formulations.

Some earlier Aroclor reference materials have been re-analysed to determine their individual CB content. These materials have been used as secondary standards to support the continuing transition from formulation-total PCB based data to the single congener analysis [44]. Maack and Sonzogni [45] have used Aroclor formulations for calibration and have then calculated the individual congeners using published data on the congener content of the specific formulation. While these data may be sound for that particular batch of the formulation, the between batch variability of these formulations is too large to reliably translate the individual CB data for use in a calibration procedure.

Williams et al. [46] have proposed the use of total CB concentrations to predict the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent concentrations (TECs) of the non-*ortho* and mono-*ortho* in fish. They concluded that this was possible within a factor of 2–5. While this approximation may be a suitable, lower cost, screening technique there are some major disadvantages of such a technique. When the TECs are close to the permitted limits then a precision of a factor of 2–5 will be insufficient and more detailed and accurate studies will be required. Also the ratio of toxic planar CBs and more persistent and abundant CBs will change between different organs within a fish, between species and populations of the same species as a result of exposure to different sources of PCB, metabolism and seasonal variations. Such CB ratios are not sufficiently constant for the detailed studies correlating planar compounds and biological effect.

The preparation, maintenance and use of the calibration solutions still remain as one of the main sources of error in the measurement of CBs. The essential precautions have been fully documented [27,29] and are summarized in Table 4.3.

TABLE 4.3

CHECK LIST FOR THE PREPARATION AND USE CB CALIBRATION STANDARDS

-
- Use pure, certified or well characterized solids when available
 - Check solvents, $\times 100$ concentration, for purity by ECD and FID or MS
 - ALWAYS check the quality of commercially available standards
 - Control the concentration of the solutions by weight
 - Check all new solutions with another reliable laboratory or prepare a second, independent, solution
 - Store calibration solutions at 0°C in sealed ampoules when possible
-

References: Wells et al. [29,30]; Duinker et al. [27].

4.5. INTERPRETATION

Since most CB data are now based upon the analysis of individual congeners, there is a vast amount of information that has to be correlated, evaluated, reported and interpreted in relation to the fate, distribution and environmental significance of these compounds.

Duinker et al. [47] normalized the data, calculating the percentage contribution of each CB to compare the distribution of 17 congeners in different organs and marine mammal species. Zitko [48] re-analysed these data using principal component analysis (PCA) to produce a more distinct and clearer evaluation, by reducing the six bar graphs and two tables to two overview diagrams. A number of workers [49–52] are now using PCA to assess the biomagnification and sources of PCBs and their distribution in different populations. This powerful technique reduces related data to a simple representation of the key differences and similarities in the CB data set (Fig. 4.2). It is interesting to note that the success of such a technique is often enhanced by data on a large set of congeners rather than a restricted number of CBs.

4.5.1. Electron capture detector

The response curves of the electron capture detector (ECD) to halocarbons have been fully reviewed [53]. The limitations of the linear range of the ECD are well known and documented, and most workers have attempted to calibrate the detector using the most linear portion of the response curve. However, with the recent improvement in the precision and accuracy of trace CB measurement [30], it is necessary for the calibration curve to be within $\pm 5\%$ of the true detector response. Under these circumstances, it is more appropriate to regard the detector as having a non-linear response and making the calibration accordingly. Single point calibration does not have sufficient accuracy and bracketing calibration points, covering the upper and lower end of the working range, are only valid if the measurement is made in the more linear portion of the ECD response curve (Fig. 4.3). A substantial proportion of environmental sample extracts contain CBs at the ultra trace level which only register in the non-linear region of the detector. The measurement of the non-*ortho* and mono-*ortho* chloro CBs at the ultra trace level along with the more

abundant congeners will span a significant proportion of the ECDs working range. Under such circumstances, it is necessary to use a multi-point calibration to reduce the errors which can occur, particularly at lower concentrations where the deviation from linearity is the greatest (Fig. 4.3). With the stability of modern GC instrumentation and detectors it is, a priori, sufficient to establish a multi-point calibration twice a week in preference to using a single or two point calibration on a daily basis. The calibration can be verified with each batch of samples (ca. 10) by including one calibration solution at approximately the same concentration as anticipated in the samples. If the check calibrant is >5% from the expected value, the detector should be recalibrated. This calibration schedule allows the response/mass profile to be maintained over a wider range without an extensive reduction of sample through-put.

To maintain this level of stability of the calibration, the ECD must be optimized at the correct operating temperature and be thermally stable. It must also remain uncontaminated from less volatile material which can gradually elute from the column. Normally, the higher detector temperature (320–340°C) will be sufficient, but when contamination increases the baseline signal of the detector, it can be cleaned in situ by replacing the make-up gas with hydrogen at a temperature of ca. 400°C for 30 min.

Lower flow rates for the make-up gas can give a substantial increase in sensitivity, however, the response becomes increasingly non-linear at these low flows [54]. This may not be so obvious from a simple plot of response against mass, but is clear when a response/mass versus mass plot is made (Fig. 4.4).

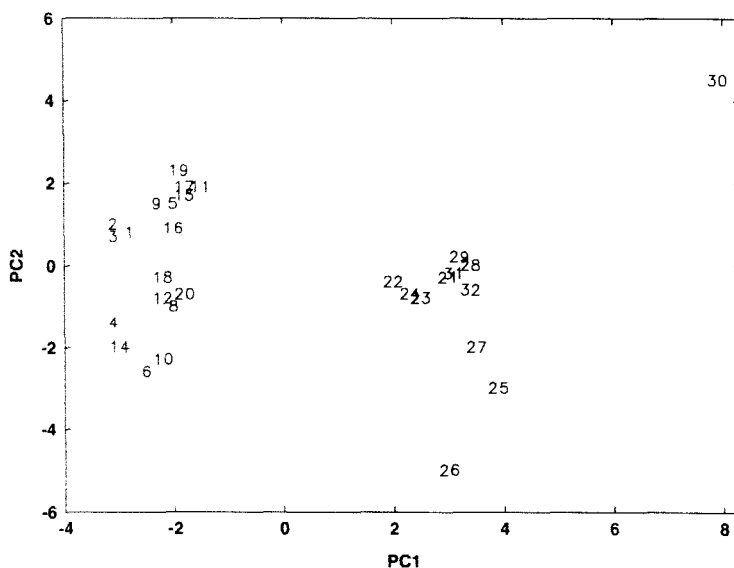


Fig. 4.2. Principal component projection of PCB in porpoise and whales [47,48]. Porpoise blubber (1–4), heart (5–8), kidney (9–12), liver (13–16) and flesh (17–20); whale blubber (21–24), heart (25), kidney (26, 27) and flesh (30–33). Reprinted with permission from [48].

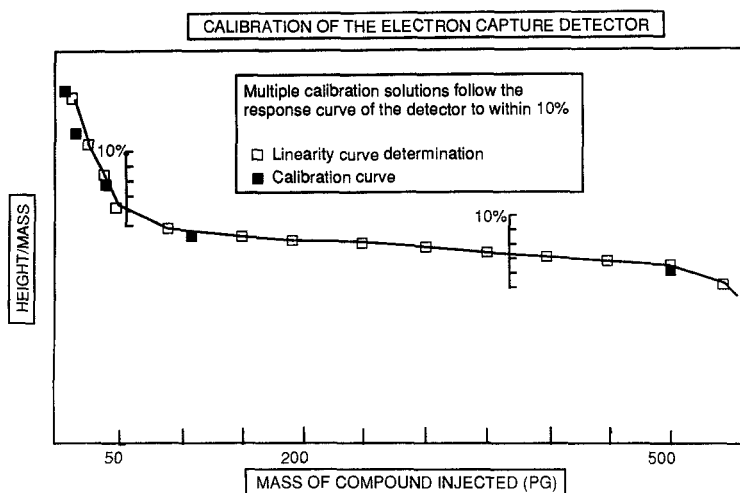


Fig. 4.3. The response of the electron capture detector is non-linear, and inappropriate calibration procedures can account for much of the errors affecting the accuracy of measurement at the trace level. This is overcome by obtaining a linearity plot of response/mass against mass injected. Calibration points can then be selected to describe the true detector response.

4.5.2. Mass spectrometry

The BCR CB intercomparison programme between 1982 and 1985 [31] concluded that, whilst MS gave additional specificity to the identification of the CBs, the measurements were not sufficiently precise for certification purposes. However, recent improvements in MS hardware and sensitivity and selectivity, particularly in the negative ion chemical ionization (NICI)-MS [55–59] mode, have made this detector considerably more amenable to the identification and measurement of CBs at the trace level and with similar precision as the ECD.

The similarity of most CB mass spectra sometimes decreases the specificity of the technique in identifying each congener, particularly when GC peaks are unresolved. Roos et al. [56] were able to identify a second CB with electron impact (EI) and NICI-MS under the peak of CB 138 which could not be separated on the more commonly used phases, e.g. CPSil 5, CPSil 8, or CPSil 19. They tentatively assigned the identity of the peak as CB 163, which has a similar retention time to CB 138 on these columns [60]. This was confirmed by Larsen and Riego [61] after they synthesized the CB 163 and were able to separate these two congeners on a very polar bis-cyanopropylphenyl phase (SP-2330). However, CB 138 also co-elutes with CB 158 on this polar column [7]. These studies have confirmed that most data reported on CB 138 is, in fact, a composite value for CB 138 plus CB 163. Since both congeners are relatively persistent, and relatively insensitive to environmental modification, most workers will continue to determine the sum of these congeners until a routine separation is available.

Tuinstra et al. [62] use high resolution MS (resolution 8000) and PFK lock mass in the electron impact (EI) mode to determine the planar, non-*ortho* chloro CB 77, CB 126 and CB 169 in animal fat. They made novel use of CB 153 as an internal standard since the endogenous CB 153 is removed during the porous graphitic carbon clean-up. The PFK lock mass at m/z 316.9824 was used to maintain the accurate mass marking and prevent mass drift, but it was also invaluable in determining any potential interferences. The advantages of high resolution MS in selectivity and sensitivity are well known. However, one disadvantage of this technique is that co-eluting material, although not positively detected by the selected mass, will competitively reduce the ionization of the determinand

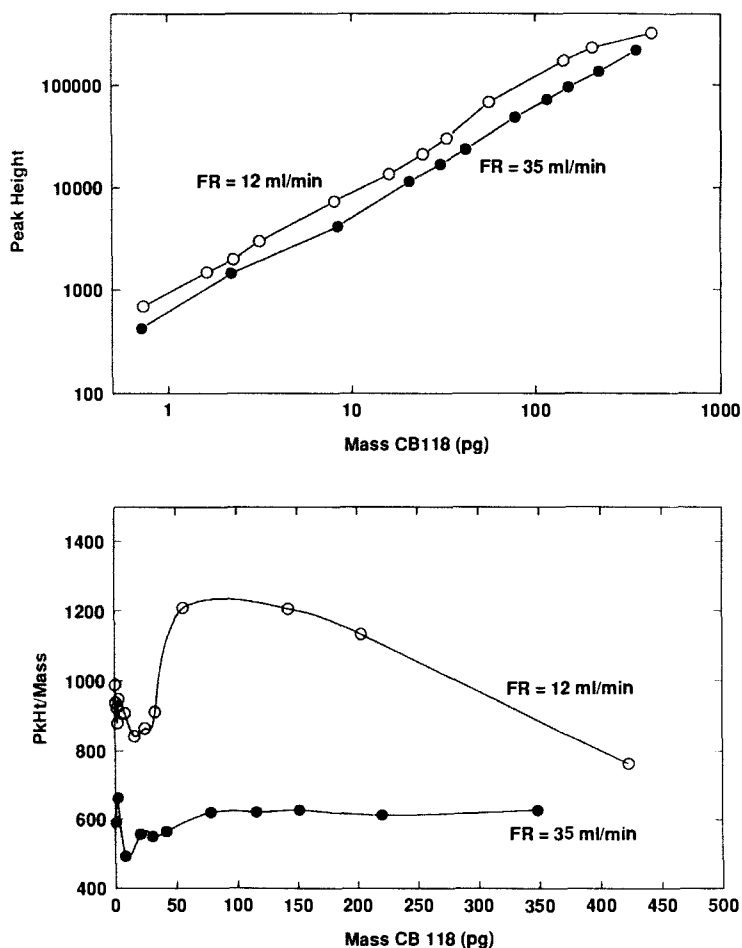


Fig. 4.4. The effect of flow rate make up gas upon the linearity and linear range of the detector. At low flow rates the linearity is considerably reduced. This is not always evident on the simple mass versus response curve, but it becomes more obvious if the response/mass is plotted against mass injected.

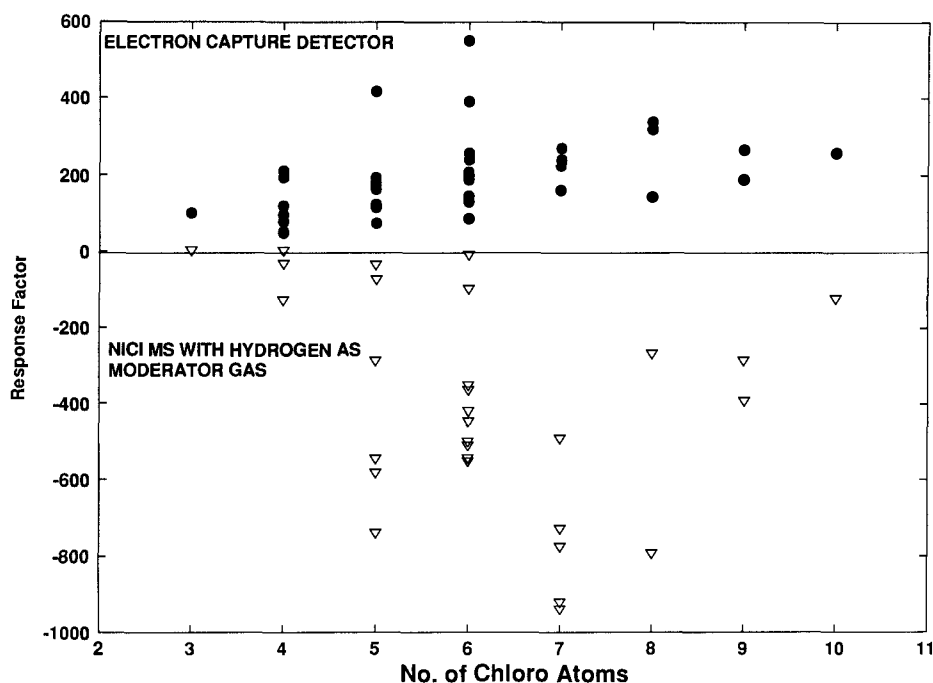


Fig. 4.5. Effects of undetected co-elutants on reproducibility. The upper trace of an NICI-MS chromatogram of non-*ortho* CBs would appear to be free from impurities. However, the selectivity of the detector hides the effect of the phthalates on CB169 (lower trace) unless observed in the EI mode. Upper trace: NICI, H_2 = 1.8 Torr, ee = 230 eV, ec = 300 μ A; Source temperature = 150°C. Lower trace: EI, ee = 70 eV, ec = 300 μ A; source temperature = 130°C. Hewlett-Packard MS engine.

within the ion source and so decrease the signal of the CB. When the lock mass is used, this interference can be detected since the PFK signal, which should remain constant, also declines.

A similar interference occurred in this laboratory during a quality check on a calibration solution of the non-*ortho* chloro CBs. The low resolution quadrupole NICI-MS total ion trace gave a higher variance for CB 169, which was trace to a phthalate impurity when the solution was re-analysed in the EI mode (Fig. 4.5).

NICI-MS has the advantage that many of the CB response factors (RFs) are comparable with the ECD. Prior to NICI-MS being available, all ECD samples were concentrated, often to very small volumes, for analysis by MS-EI or positive ion-chemical ionization (PICI-)MS. This degree of sample concentration had the added disadvantage that solvent impurities became apparent and that other compounds with a similar MS response would mask the CB, making confirmation difficult.

A comparison of the RFs for 33 CBs obtained by ECD and NICI-MS is given in Fig. 4.6. Wells et al. [54] used hydrogen as the moderator gas to generate the negative ions

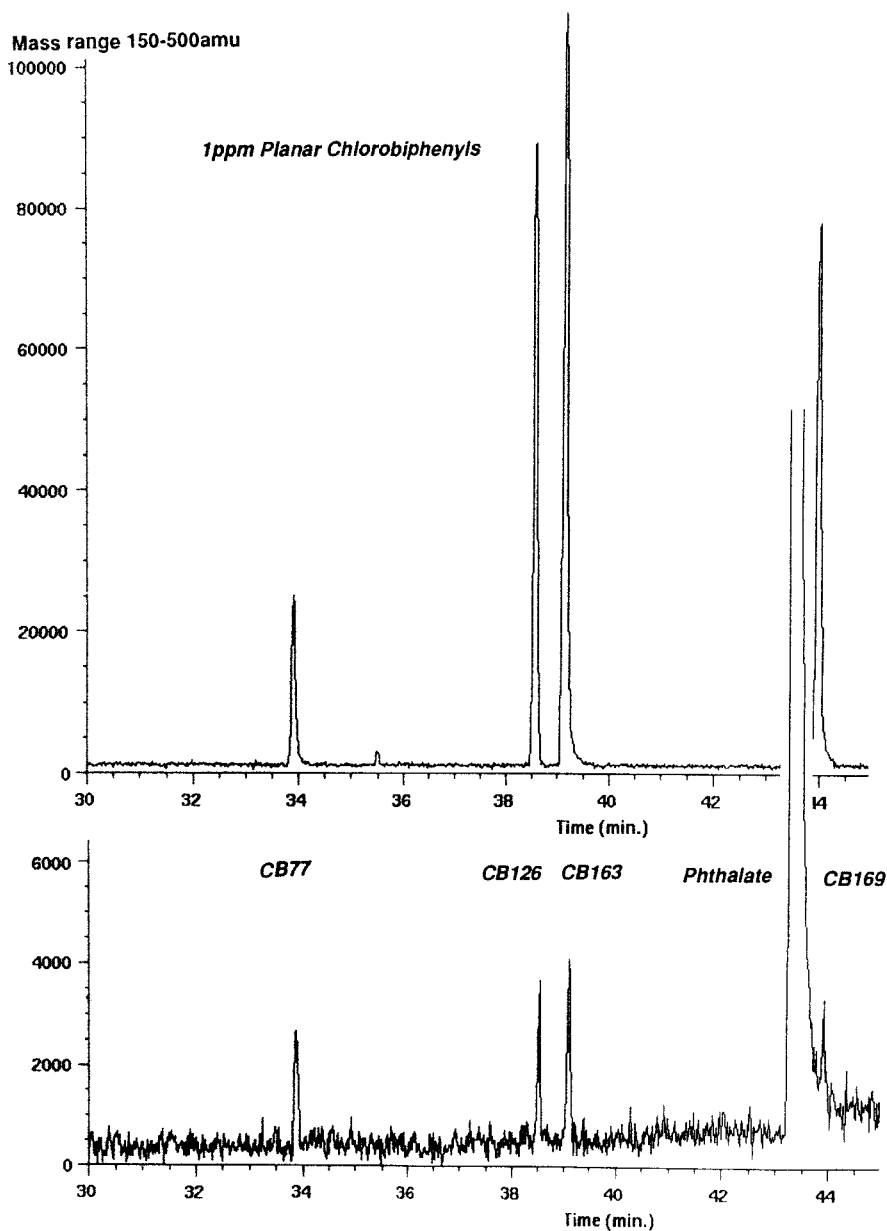


Fig. 4.6. Response factors of chlorobiphenyls using the ECD and NCI-MS. Most congeners with more than four chlorine atoms have similar values with both the NCI-MS and the ECD. The difference in response between the two detectors increases significantly for $\text{Cl} < 4$. There are some specific exceptions. See Fig. 4.7.

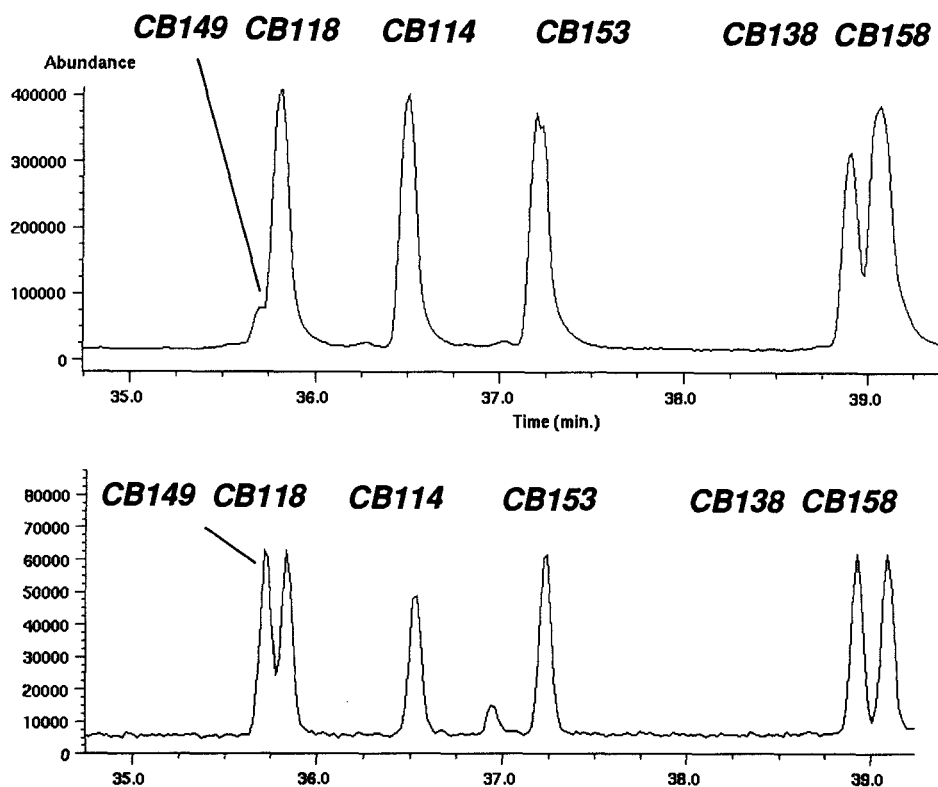


Fig. 4.7. Chromatogram for CB149 and closely eluting congeners in EI and NCI-MS mode. There is no increase in sensitivity for CB149 in the NCI-MS mode relative to all other CBs with a similar number of chlorine atoms. The selectivity of NCI is primarily affected by the cross sectional area of the molecule.

with a two-fold increase in sensitivity for the CBs compared with methane. Other advantages of using the hydrogen are: (a) a single gas system; (b) a cleaner ion source for considerably longer periods; (c) a similar carrier gas. The NCI-MS RFs for the congeners with less than 3/4 chloro atoms are substantially lower than more chlorinated CBs, but the response is not dependent on the number of chlorine atoms alone. Quite small changes in structure can also significantly change the RFs. CB 149, 2,3,6,2',4',5'-hexachlorobiphenyl, had an ECD RF of 256 which was drastically reduced to 11 by using NCI-MS. By comparison CBs 151, 2,3,5,6,2',5'-hexachlorobiphenyl has the same number of chloro atoms as CB 149, but with the chloro in the 4' position moving to the 5 position on the first ring. Here CB 151 has an ECD RF of 145 and a NCI-MS RF of 355 (Fig. 4.7).

Patterson et al. [36] used isotope dilution MS (IDMS) to determine non-*ortho* chloro and *ortho*-substituted CBs in human serum. They synthesized over 50 ^{13}C -labelled CBs normally found in human tissue and determined each CBs using either EI or NCI-MS. The analytical coefficient of variation was estimated to be between 12 and 18% at the parts-per-quadrillion level. Although the recoveries of CB 126 and CB 169 were low, the

isotope dilution technique compensates for this in each sample giving an acceptable estimate of these congeners in the serum. The recoveries for CB 77 were, however, falsely high due to interferences from the carbon column clean-up.

Kuehl et al. [63] used high resolution IDMS to determine the planar CBs in fish and marine mammals. They also used the ^{13}C -labelled CBs 77, 126 and 169 as internal standards, but in addition ^{13}C -labelled CB 101 was used as a recovery standard. CBs 136, 110 and 77 were used to check the resolution of the GC column, since these congeners form a closely eluting triplet on the DB 5 column.

The MS has often been regarded as a selective and sensitive filter to exclude interferences from co-extracted materials while accurately measuring trace components, such as planar CB. This is often not the case as co-extracted material can seriously affect the determinant response. Selectivity should never be a substitute for separation. Adequate low resolution (clean-up and group separation) and high resolution, single column or multi-dimensional, chromatography are essential precursors to selective measurement of toxic CBs at the ultra trace level.

4.6. CHROMATOGRAPHIC SEPARATION

High resolution capillary column GC separation is normally the final stage in the isolation of each CB prior to the final determination by ECD or MS normally. The selection of the capillary column's physical parameters, stationary phase and GC conditions are crucial in obtaining a single uncompromised signal resulting from the elution of one congener, free from interference from other CBs and co-extracted material. It is appropriate to remember that the GC-ECD is not a confirmatory technique.

4.6.1. Optimization of the high resolution gas chromatograph (HRGC)

A number of groups have studied the optimal conditions for the separation of those CB which are most commonly determined in environmental samples [5,9,29,61,64–67]. A summary of the key conclusions of those studies is given below.

The overall optimization of the GC is essential if the maximum column efficiency is to be obtained [29,31]. In addition to the optimal sample introduction and column installation, the capillary column separation of the CBs is dependent on (a) carrier gas, (b) column parameters and (c) choice of stationary phase(s).

Preferably, hydrogen should be used as the carrier gas with a linear velocity of ca. 40 cm s^{-1} . The second choice of carrier gas would be helium with a linear velocity of ca. 25 cm s^{-1} . Nitrogen should not be used as it drastically reduces column efficiency, and increases the analysis time with no obvious advantages. Higher linear velocities should not be used in an attempt to reduce the analysis time, especially with the longer columns ($>25\text{ m}$). The higher velocity will simply negate the effect of the additional column length, although the loss of resolution is less critical if hydrogen is used as carrier gas.

The column length should be 50 m or more to obtain the necessary separation on most available phases unless multi-dimensional gas chromatography (MDGC) is used. Narrow bore columns (0.22 mm i.d.) with a stationary phase film thickness of ca. $0.22\text{ }\mu\text{m}$ are

normally required to achieve the required separations between some of the closely eluting congeners. Shorter columns (25 m) with a wider bore (0.32 mm i.d.) do not give the required degree of separation between some closely eluting congeners [29,64,66].

4.6.2. Selection of chromatographic columns

The only complete set of retention time data so far available for all 209 congeners was obtained by Mullin et al. [60] for the SE 54 5% diphenyl 1% vinyl dimethyl siloxane stationary phase and, for this reason, most analyses separation have been based on columns with this phase. However, additional GC retention data have become available for a number of CBs on different columns. De Boer et al. [66] measured the retention times of 51 CBs on seven narrow bore columns, CPSil 8, CPSil 12, CPSil 19, CPSil 88 and C18 from Chrompack, SB Smectic from Lee Scientific, and the FFAP from Hewlett Packard. Bowadt and Larsen [67] coupled a 50 m \times 0.25 mm i.d. (0.26 μ m film thickness) CPSil 8 with a 25 m \times 0.22 mm i.d. HT-5 column. The HT-5 is a newer column coated with 1,2-dicarba-*closo*-dodecarborane polydimethylsiloxane which is a high temperature ($>300^{\circ}\text{C}$) material based on the "Dexil 410" packed column liquid phase. Over 84 CBs were resolved by ECD and 108 CBs by MS using this coupled system. The separations and peak assignments were given, but no retention data. Larsen et al. [68] made a comparison of the separation of the toxic CBs from the potentially interfering CBs on eight different phases and a further study [69] of the separation of 140 CBs in technical mixtures on five different phases with data on all potentially overlapping peaks. A summary of these data for the key toxic and monitoring CBs is given in Table 4.4.

4.6.3. Multi-dimensional chromatography

Every attempt to optimize the capillary GC separation conditions to unambiguously separate all 209 CBs on a single column of either single or mixed phases has had limited success. Alternative phases to the SE 45 do separate different groups of congeners with improved resolution for some CBs, but with a loss of resolution between other congeners [70]. The lack of retention data for every CB on these alternative phases has been an additional limitation and always leaves some room for doubt regarding the identification of some congeners. Multi-dimensional GC (MDGC) has been used successfully to clarify the identity of CBs in industrial mixtures [5,71] and in environmental samples [65,72] and to solve many of the questions relating to the relative amounts of the CBs. Although MDGC is primarily a qualitative research instrument there will always be a requirement for a technique to provide this level of resolution for the identification of co-extracted unknown compounds, particularly at the ultra trace level of the planar CBs.

The present configuration of the MDGC [73,74] normally uses a non-polar phase on the first column such as SE 54 or CPSil 8 to make the initial, well characterized separation. The sample is chromatographed on this column to a point just prior to the elution of the unresolved peaks. The column flow is then switched into the second column of a different, usually more polar, phase, e.g. CPSil 19, CPSil 88, for the duration of the elution of these unresolved peaks only. The columns are again isolated and the small group of

unresolved peaks are separated on the second column. The inherent resolving power of the system is obtained by coupling of two columns of different polarity and isolating relatively few compounds on the second column. This technique is ideal for specific separations for identification purposes.

The main disadvantage of quantitative MDGC analysis is that measurements are only made for a small number of unresolved compounds, and the determination of all selected CBs, with their surrogate and internal standards, has not been fully optimized for this technique.

Schulz et al. [5] re-examined commercial Aroclor and Clophen industrial formulations of PCB using MDGC to completely characterize the CB content above the 0.05% level. Each PCB mixture was initially chromatographed on an SE 54 and the chromatogram separated into distinct regions containing one or two peaks which were individually heart-cut into the second OV 210 or C-87 column. Some of the unresolved peaks on the SE 45 would be better resolved on a narrow bore, rather than an 0.32 mm i.d. column, but the MDGC information is extremely valuable since it specifically confirms the absence of 77 CBs from these formulations above the 0.05% level. The combination of these data with the retention data obtained by Larsen et al. [69] for the 140 CBs on five different stationary phases give a complete set of retention data for all CBs on all five phases, with the exception of CBs 69, 75, 96 and 182.

TABLE 4.4

CO-ELUTING CBS ON CAPILLARY GC COLUMNS WITH DIFFERENT STATIONARY PHASES

Congener	HT-5	CPSil 5	CPSil 8	CPSil 19	CPSil 88
28	—	—	—	—	16
52	—	—	—	—	—
77	149	—	110	—	82/183/187
101	60	—	84	—	55
105	141	132	132	—	129
118	—	—	149	—	200/123
126	167/185/202	129	129/178	—	—
128	159/174	—	167	—	193/201
138	—	160/163	160/163	160/163/158	—
153	—	—	—	—	—
156	172	171	202/171	—	—
157	—	202	173/200	180/197	—
158	175/178	—	—	163/138	138/160
169	—	—	—	203/196	—
170	—	—	190	190	—
180	193	—	—	197	197
194	—	—	—	—	—

References: Larsen [68]; Larsen et al. [69]; Larsen et al. [122].

De Boer and Dao [72] used MDGC to study the potential interferences in the seven monitoring CBs selected in 1982. They confirmed that CB 138 and CB 163 co-elute on CPSil 8 as does CB 84 and CB 101. The first pair can be effectively separated on HT-5, without any interference from other CBs similar separation can be made for CB 84 and CB 101 on CPSil 5 or preferably CPSil 19.

MDGC is not a widely available technique and may remain a valuable research tool for some while. However, the information which this technique has made available allows all CBs to be determined without any ambiguity from other CBs on five different stationary phases.

4.7. GROUP SEPARATION

Normally, it is necessary to make a series of group separations prior to the final resolution of the CBs and organochlorines by HRGC. The cleaned-up extract, at this stage, will contain other organohalogens such as organochlorine pesticides (OCPs), polychlorinated naphthalenes (PCNs), polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzofurans (PCDFs), polychlorinated camphenes (PCCs) (toxaphene) as well as the CBs [6]. The OCPs and the more abundant CBs are normally the predominate two groups of OCs, and these can easily be separated on silica gel [75,76] and determined separately. Most of the PCCs can also be separated from the CBs and the OCPs on the silica gel by increasing the polarity of the solvent from a *n*-alkane to between 5 and 15% methyl tertiary butyl ether in hexane [75]. This separation is normally adequate when the predominant monitoring CBs are to be determined.

However, with the exception of CB 118, and to a lesser extent CB 105, all mono-*ortho* CBs and the non-*ortho* CBs are present at substantially lower concentrations compared with the remaining CBs. It is, therefore, necessary to separate the non-*ortho* and mono-*ortho* chloro CBs into different groups since: (i) the range of concentrations of the CBs is normally too large for all congeners to be measured without additional dilution or concentration; (ii) some of the key CBs are not resolved from each other on a single GC column, regardless of the column phase.

The methods available for the isolation of the CBs into separate fractions, prior to GC analysis, utilize the spatial planarity of these compounds.

4.7.1. Adsorption columns

Storr-Hansen and Cederberg [77] investigated the use of adsorption chromatography to separate the non-*ortho* chloro CBs from the remainder of the CBs. The initial work on florisil reported by Kamops et al. [78] and more recently by Fernandez et al. [79] was repeated with the inclusion of alumina and silica in these studies. Normally these absorbents are adjusted to a specific activity with water in order to remove co-extracted materials from soil, sediment or tissue matrices. In this case Storr-Hansen and Cederberg [77] heated the adsorbents at 450°C to fully activate the material which was used without further deactivation. A mixture of CBs, including the non-*ortho* chloro congeners, was re-applied to a column (200 × 10 mm² i.d.) containing activated basic alumina (6 g) and

eluted with hexane. Two observations were made. Firstly, the elution volume for the bulk of the CBs increased from around 10–20 ml to 150 ml, and secondly, the planar congeners, CB 37, CB 81, CB 77, CB 126 and CB 169 were only partially eluted after some 300 ml. The similar studies with active florisil gave approximately the same pattern of retention of the planar CBs, but with a smaller elution volume. The elution pattern with activated silica did not drastically alter from the normal deactivated (ca. 3–5%) adsorbent with the CBs eluting with 50 ml of hexane. Storr-Hansen and Cederberg [77] concluded that the lack of selectivity by the SiO₂ was due to the different –OH bonding. An alternative explanation is also possible. Highly activated silica is very hygroscopic and even when the column is prepared rapidly at room temperature, the adsorbent will become deactivated to at least 1% due to the surrounding water vapour in the laboratory atmosphere. When the alumina and florisil were deactivated to >3% with water the separation between the planar and nonplanar CBs was completely lost and all CBs are eluted with 20–30 ml hexane.

Harrad et al. [80] also used florisil, activated to 130°C for 16 h, to separate the planar CBs from the *ortho* chloro CBs. The sample was added to a 1-g column in a pasteur pipette and first eluted with hexane (10 ml) to remove the bulk of the CBs and then eluted with dichloromethane to isolate CB 77, CB 126, and CB 169. Recoveries ranged from 78% to 107% with accuracies of between 26 and 33% for spiked samples which had been previously cleaned-up with sulphuric acid. This method was applied to human milk, adipose tissue, avian tissue, soil and sewage sludge using ¹³C analogs with the final determination by IDMS.

These adsorption methods are suitable for the separation of the planar CB from the other *ortho* chloro CBs only and the matrix must be thoroughly cleaned up prior to this separation being made. Normal lipid removal cannot be made simultaneously on the same column.

4.7.2. Activated carbon

Activated carbon has been used extensively to separate the non-*ortho* chloro and the mono-*ortho* chloro CBs from the remaining congeners [9]. The early attempts at using carbon columns were directed towards the separation of the PCDDs and PCDFs from other organochlorines and are covered in the review by Erickson [4]. In 1974, Jensen and Sundstrom [1] used an activated charcoal column (200 × 15 mm² Darco G-60) to separate the four groups of PCBs in Clophen technical mixtures. The tetra-*ortho* chloro CBs, Group 1 and the tri-*ortho* chloro CBs, Group 2 were eluted with tetrahydrofuran. The mono- and di-*ortho* chloro CBs, Group 3, and the non-*ortho* chloro CBs, Group 4 were eluted with benzene. Following this, some workers used polyurethane foam to disperse the activated carbon. Huckins et al. [24] and Stalling et al. [81] used AMOCO PX-21 15% w/w on the polyurethane foam. Stalling eluted the CBs into five fractions from tetra to non-*ortho* chloro CBs with a step gradient of toluene/cyclohexane (2:98–100:0).

Polyurethane foam support for activated charcoal has had limited success due to the breakdown of the foam with polar solvents. Glass fibre was used as an alternative substrate for the activated carbon [32,82], with the planar molecules being trapped on the

carbon from the extract in dichloromethane/benzene (1:1) and backflushed from the column with toluene.

Tanabe et al. [35] reverted to using a simple activated carbon column. After removing the co-extracted lipophilic material by saponification, they adsorbed the OCs onto the carbon column from the hexane extract. The OCPs and non-planar compounds were eluted with dichloromethane/hexane (1:5), the non-*ortho* chloro CBs were eluted with benzene/ethyl acetate (1:1) and the PCDDs and PCDFs with toluene. The recoveries for CB 77, CB 126 and CB 169 were >90% at the $10 \mu\text{g kg}^{-1}$ level and >60% at the $0.1 \mu\text{g kg}^{-1}$ level.

Wilson-Yang et al. [83] used a carbon/glass fibre column ($180 \times 4 \text{ mm}^2$ i.d.) to separate the non-*ortho* chloro CBs from the *ortho* CBs. The sample was eluted under nitrogen (ca. 30 psi) with dichloromethane in hexane (1:3, 10 ml) to remove the CBs 28, 52, 60, 138, 166, 170 and 158, followed by dichloromethane to elute CBs 123, 118, 114, 157, 105, 167, 156 and 189. The flow and pressure were reversed and the planar CBs 77, 81, 126 and 169 were eluted with toluene (20 ml). The samples were spiked at levels between 70 and $200 \mu\text{g kg}^{-1}$.

Sericano et al. [84] used a 1:20 mix of activated AX-21 carbon and low pressure silica gel LPS-2. They tested the efficiency of the column with an Aroclor 1254 and a dolphin extract spiked with the planar congener at 20, 50 and $100 \mu\text{g kg}^{-1}$. The first eluate of dichloromethane/cyclohexane (50 ml) contained the *ortho* chloro CBs and the second eluate of toluene (40ml) contained the planar CBs 77, 81, 126 and 169. The recoveries were reported to be between 82 and 96%.

The attraction of using activated charcoal is that it has a high affinity for organics even at the ultra trace level, it is inexpensive, readily available and easy to use. However, there are also some significant drawbacks to its use in the present application.

It is essential to separate the mono-*ortho* chloro CBs as well as the non-*ortho* chloro CBs from the other congeners, since both groups contribute to the overall toxicity of the PCBs. The mono-*ortho* chloro CBs are present at considerably higher concentrations and therefore may contribute as much, if not more than the planar CBs to the overall toxicity, and subsequent biological effect. The recovery of planar CBs from active carbon at the level that occurs in environmental samples has not always been fully quantitative. However, low recoveries can be overcome by using IDMS, but the impurities in the carbon have proved to be a greater problem. Kannan et al. [85] made an extensive and thorough comparison of six active charcoal (Wako, Baker Analytical, Serva-SK-4, Anderson, AX-21, Alltech, SK-4 type, and Merk) types using the MDGC-ECD techniques developed by this group [5,71] to determine the efficiency and separation of these CBs. They use a test mixture (Table 4.5) containing 0-4 *ortho* chloro CBs to optimize the separation prior to testing on a batch of Aroclor 1254 which had been previously characterized [5]. With the advantage of the MDGC, it was possible to clearly identify the problems of separation with these materials. They concluded that the activated charcoals tested were not able to completely separate the non-*ortho* chloro CBs from the dominant *ortho* chloros. In particular the co-elution of CB 110 with CB 77, and of CBs 129 and 178 with CB 126 was sufficient to prevent the quantification of the planar congeners. They concluded that data for CB 77 and CB 126 obtained by using these forms of activated charcoal, without the advantage of MDGC, are likely to be an overestimate. They also found that it

was difficult to obtain a clean blank with these activated charcoals even after extensive cleaning. These impurities are a major drawback of this method which has been explicitly and comprehensively reported for the first time.

Storr-Hansen and Cederberg [77] used a Carbpak C/Celite column ($200 \times 10 \text{ mm}^2$). After cleaning the column in situ the *ortho* chloro CBs were eluted with hexane (50 ml) and the non-*ortho* chloro CBs 37, 77, 81, 126 and 169 were removed with toluene (15 ml). The planar CBs were determined by IDMS using the ^{13}C -labelled CBs with recoveries of CB 77, $79 \pm 14\%$, CB 126, $80 \pm 15\%$, CB 169, $83 \pm 17\%$, $n = 63$). The spiking level was 4 ng equivalent to 400 ng kg^{-1} in the sample. The advantage of using Carbpak C over the more conventional activated carbon has meant that the CBs can be eluted with a smaller volume of less polar solvent to remove the *ortho* chloro CBs and the non-*ortho* chloro CBs can be eluted without having to backflush them from the column.

Atuma and Andersson [86] used active coal (SP-1) on Chromosorb to separate the 2-4 *ortho* chloro CBs and 4,4'-DDE with hexane dichloromethane, and the mono-*ortho* and non-*ortho* chloro CBs by eluting with toluene. The mono-*ortho* and non-*ortho* chloro CBs were subsequently separated using Carbpak C and eluting with hexane and toluene respectively. The ^{13}C -labelled CBs were used as surrogate standards for CB 77, 126 and 169, CB 189 for the mono-*ortho* chloro and CB 53 for the 2-4 *ortho* chloro CBs.

Lundgren et al. [87] have used active carbon Amoco PX-21 incorporated into a $250 \times 4.5 \text{ mm}^2$ HPLC column. The Amoco PX-21 ($2\text{--}10 \mu\text{m}$), ca. 100 mg, was dispersed onto Lichrosphere RP-18. The solvent gradient was hexane (100%) to hexane/dichloromethane (1:1) over 150 ml at 4 ml min^{-1} . Fractions (10 ml) of the eluate were collected for separate analysis to identify the separation of each *ortho* chloro group. A total of 31 CBs were used in the initial mixture. Most of the 4-3 *ortho* chloro CBs eluted in the first 20 ml, followed by the di-*ortho* chloro (10-40 ml), mono-*ortho* chloro (40-90 ml) and the non-*ortho* chloro CBs 150-240 ml.

TABLE 4.5

SELECTION OF NON-*ORTHO* AND *ORTHO* CHLORO SUBSTITUTED CBS FOR TESTING THE SEPARATION ON ACTIVATED CHARCOAL

IUPAC No	Structure	No <i>ortho</i> Cl	Concentration (ng ml^{-1})
14	3,5	0	66
77	3,4,3',4'	0	200
126	3,4,5,3',4'	0	110
169	3,4,5,3',4',5'	0	100
105	2,3,4,3',4'	1	200
116	2,3,4,5,6	2	100
138	2,3,4,2',4',5'	2	100
171	2,3,4,2',3',6'	3	100
136	2,3,6,2',3',6'	4	100

Data in this table according to Kannan et al. [85].

4.7.3. Porous graphitic carbon

The porous graphitic carbon (PGC) HPLC packing developed by Knox et al. [88] was used to separate CBs, PCDDs and PCDFs by Creaser and Al-Haddad [89]. The PGC has surface area of $150 \text{ m}^2 \text{ g}^{-1}$ and a mean particle size of $7 \mu\text{m}$, with a pore volume of $2 \text{ cm}^3 \text{ g}^{-1}$ which is ideal for use in an HPLC system. The OCs were chromatographed on the column ($50 \times 4.7 \text{ mm}^2$ i.d., Shandon Southern, UK) with *n*-hexane. The OCPs and *ortho* chloro CBs were eluted in the first 10 ml using a flow rate of 1 ml min^{-1} . The non-*ortho* chloro CBs were eluted with a further 90 ml of hexane. The column was backflushed with a further 200 ml of hexane to remove the PCDDs and PCDFs. The backflush volume can be reduced by using toluene in place of hexane. The advantage of such a system is that the *ortho* and non-*ortho* chloro CBs can be separated using a single solvent with an HPLC system that can be fully automated.

Tuinstra et al. [62] used the same PGC "Hypercarb" column, but with a solvent mix of dichloromethane/cyclohexane (1:1) at 2 ml min^{-1} to separate the toxic CBs in animal fat. The increase in polarity reduced the elution volume of the CBs. The first fraction contained the *ortho* chloro CBs. The solvent was then switched to toluene to remove the non-*ortho* chloro CBs, again to reduce the elution volume. The column was completely washed free of toluene with the starting solvents prior to the next sample.

Hong et al. [38] used the PGC column to separate the non-*ortho* chloro CBs in human milk. They used the same single hexane solvent as Creaser and Al-Haddad [89], but reversed the flow after the elution of the *ortho* chloro CBs to speed up the recovery of the second fraction.

Zebühr et al. [90] used two coupled HPLC columns to improve the isolation of the CBs according to their *ortho* chloro substitution pattern. They coupled an aminopropyl RP column ($250 \times 10 \text{ mm}^2$) with a Hypercarb column ($100 \times 4.7 \text{ mm}^2$). All extracts of fish tissue were cleaned up prior to this HPLC separation to remove the lipids. The samples were eluted with *n*-hexane through the amino propyl column to isolate the aliphatics and mono-aromatics, e.g. hexachlorobenzene. The second fraction containing the diaromatics PCDD/Fs, PCNs and CBs, was switched to the second Hypercarb column. The polyaromatics were removed from the first column by backflushing. The diaromatic fraction were firstly eluted from the PGC with hexane (4–2 *ortho* chloro CBs) and then with hexane/dichloromethane (1:1) to remove the mono-*ortho* chloro CBs. The PGC column was washed with dichloromethane/methanol (1:1) and then backflushed with toluene at 40°C to isolate the PCDD/FS and the non-*ortho* chloro CBs together.

4.7.4. Pyrenyl silica column

The activated carbon and the porous graphitic carbon is complemented by the silica bond phase, 2-(1-pyrenyl) ethyldimethylsilylated (PYE) silica [91,92] which can separate the *ortho* and non-*ortho* chloro CBs on the basis of the degree of planarity and chlorination. This column material separates structurally similar molecules with different π -electron densities resulting from the spatial configuration of the aryl rings, and has sufficient resolution to isolate the non-, mono- and other *ortho* chloro CBs. Initially, this type of column was, like the activated carbon and the PGC, used to separate the toxic CBs 77,

126 and 169. However, it is also possible to separate other key CBs which can co-elute on the 5% phenyl methyl GC column (SE 54, CPSil 8 type) (Table 4.6). This column, therefore, has the potential to remove a number of ambiguities that sometimes occur in the final determination of the toxic CBs where MDCG is not available. Haglund et al. [92] have used this column to determine the mono- and non-*ortho* chloro CBs in reindeer, herring and seal tissue and Wells and Echarri [7] have measured a similar suit of CBs in seal, dolphin, porpoise and whale with a similar separation technique.

Using the retention pattern of 168 CBs available on this PYE column [7,91,93] it has been possible to isolate three different fractions which will isolate predominantly the tri- and di-*ortho* chloro CBs (fraction I) from the mono-*ortho* (fraction II) and the non-*ortho* chloro CBs (fraction III). The elution order of the CBs is not solely dependent upon the degree of *ortho* chloro substitution. A number of the more highly chlorinated CBs elute in the second and third fractions. For example, the effect on retention of the *ortho*-substitution with the more highly chlorinated CBs is seen by comparing CBs 205, 206 and 209, where one aryl ring is fully substituted. The second ring has a 3',4',5' pattern for CB 205, which elutes in fraction III. By adding one further *ortho* chloro atom to the ring, the elution of CB 206 now becomes split between fractions I and II, and the fully substituted CB 209 with the last *ortho* chloro position substituted elutes completely in fraction I [7].

These fractions separate pairs of CBs which have similar retention times on an SE 54-CPSil 8 type column and overcome some of the problems encountered by Kannan et al. [85] using the activated carbon columns without MDGC (Table 4.6). CB 129 and CB 178 which normally co-elute with CB 126 are separated into fractions I and III, and CB

TABLE 4.6

THE SEPARATION OF CBS ON A PYE HPLC WHICH ARE NOT FULLY RESOLVED ON AN SE54 PHASE GC COLUMN

IUPAC No	Fraction from the PYE HPLC column
77	III
110	II
126	III
129	I
178	I
138	I
163	II
153	I
132	I
105	II
156	II
171	I
202	I

References: Haglund et al. [91]; Haglund et al. [92]; Wells and Echarri [7,93].

110 (fraction II) is fully separated from CB 77 (fraction III). The two CBs 138 and 163 are also separated into fractions I and II, respectively [7], although a full 100% split is difficult to maintain since any small shift in retention times of 3–5 s can result in ca. 5% of the more abundant CB 138 in fraction II.

The retention times using the pyrenyl column are very repeatable within a batch calibration, but are susceptible to change if the sample contains any lipid residues. Both Haglund et al. [92] and Wells and Echarri [7] found it essential to remove all lipophilic co-extracted materials prior to separating the CBs on the PYE column. The column efficiency can be maintained by regular flushing of the column with ethyl acetate between sample batches [7,93].

The PYE HPLC column is ideally suited for separating organics on the basis of their planar structure. Wells and Echarri [7,93] investigated the elution profile of other groups of planar compounds that have AHH/EROD activity and can also interfere with the determination of the toxic CBs. These were the polynuclear aromatic hydrocarbons (PAHs), the PCNs, the PCDDs and PCDFs. The capacity factors of each of the compounds chromatographed on this stationary phase are given in Table 4.7, and a comparison of the separation of these groups is given in Fig. 4.8.

Like the activated carbon and the PGC, the PCDDs and the PCDFs can be separated from the CBs on the PYE column. However, it is also possible to separate the PCDDs and the PCDFs into their own fraction according to the degree of chlorination (Fig. 4.8). In view of the high k' values for the hepta and octa chloro isomers, it is preferable to remove these by backflushing or by increasing the polarity of the solvent.

The potential interference from PCN can also be reduced with the PYE column. The penta and hexa CNs elute in fraction III along with the non-*ortho* chloro CBs. But the predominant CNs which occur in the PCN formulations such as the Halowax 1041 (56% Cl) are separated by GC from the planar CBs (Fig. 4.9) [93].

4.8. CLEAN-UP

The chromatographic materials used to isolate the planar CBs are highly sensitive to trace amounts of lipophilic material which affect the active surfaces of the stationary phase and degrade the resolving power of the column. An effective clean-up procedure is therefore, essential. Since the toxic CBs are present in most samples at the ultra trace level, the sample mass required is normally larger than that which is used for measuring the routine monitoring CBs. It follows, therefore, that the amount of lipophilic material in the extract will also be much greater.

Extracts containing ca. 250 mg of lipid from biological tissue or 50 mg from sediment is usually sufficient to determine the more abundant CBs in all but the cleanest samples. However, the larger sample size for the planar CB analysis can result in extracts containing up to 10–20 g of lipid, all of which must be efficiently removed prior to further sample treatment [7,9,35]. Traces of lipid, e.g. 1 mg may only be a fraction of the total extract (ca. 1:10 000) but this will become very significant when the sample is concentrated to 1 ml. This amount of lipid remaining in the extract will degrade a carbon or pyrenyl column when separating the planar CBs. Fortunately, most CBs are relatively robust

TABLE 4.7

CAPACITY FACTORS (k') FOR PLANAR MOLECULES ON THE PYRENYL HPLC
COLUMN ELUANT:HEXANE, 1 ml/min

	k'		k'
PAHs			
Pyrene	0.61	2,3,4,8 TCDF	3.91
Benzo(<i>b</i>)naphtho(2-1- <i>d</i>)thiophene	0.82	2,3,7,8 TDCF	4.50
Benzo(<i>b</i>)naphtho(1-2- <i>d</i>)thiophene	0.82	1,2,3,8,9 PCDF	8.28
Benzo(<i>a</i>)anthracene	0.91	1,2,3,7,8 PCDF	8.29
Chrysene	0.97	2,3,4,7,8 PCDF	13.64
Triphenylene	1.09	1,2,3,7,8,9 HCDF	16.88
Benzo(<i>k</i>)fluoranthene	1.29	1,2,3,4,8,9 HCDF	18.89
Benzo(<i>b</i>)fluoranthene	1.44	1,2,3,4,7,8 HCDF	21.29
Benzo(<i>a</i>)pyrene	1.56	1,2,3,6,7,8 HCDF	21.76
Benzo(<i>e</i>)pyrene	1.66	1,2,3,4,6,7,8 H7CDF	64.62
Perylene	1.79	1,2,3,4,6,7,8,9 OCDF	145.62
Indeno(1,2,3- <i>cd</i>)fluoranthene	2.05	PCBs	
Indeno(1,2,3- <i>cd</i>)pyrene	2.30	CB 28	0.42
Benzo(<i>g,h,i</i>)perylene	2.65	CB 52	0.42
		CB 101	0.45
PCDDs			
1,3,6,8 TCDD	3.31	CB 149	0.48
1,2,3,4 TCDD	3.44	CB 153	0.49
1,3,7,9 TCDD	3.48	CB 138	0.63
1,2,3,7 TCDD	3.54	CB 180	0.63
1,3,7,8 TCDD	3.61	CB 118	0.70
1,2,7,8 TCDD	3.67	CB 163	0.72
1,2,3,8 TCDD	3.73	CB 128	0.73
1,2,8,9 TCDD	3.94	CB 105	0.80
2,3,7,8 TCDD	4.29	CB 170	0.81
1,2,8,7 TCDD	5.00	CB 156	1.00
1,2,6,7 TCDD	5.00	CB 77	1.20
1,2,3,4,7 PCDD	8.20	CB 126	1.74
1,2,3,7,8 PCDD	9.02	CB 169	2.22
1,2,3,4,7,8 HCDD	19.27	Chloronaphthalenes	
1,2,4,6,7,8 HCDD	21.01	DiCNs	0.37
1,2,3,7,8,9 HCDD	21.01	TriCNs	0.62
1,2,3,4,6,7,8,9 OCDD	172.46	TriCNs	0.74
		TriCNs	0.93
PCDFs			
1,2,7,8 TCDF	3.36	TriCNs/TetraCNs	1.24
		1,2,3,4 TCN	1.25

TABLE 4.7 (continued)

	k'		k'
TetraCNs	1.36	HexaCNs	5.96
TetraCNs	1.77	HexaCNs	6.46
PentaCNs	2.47	HexaCNs	7.54
PentaCNs	2.64	HexaCNs	8.68
PentaCNs	2.92	HeptaCNs	9.31
HexaCNs	3.21	1,2,3,4,6,7,8,9, OFN	17.36
HeptaCNs	3.35	2 Bromonaphthalene	0.39
HeptaCNs	3.76	2 Bromoanthracene	0.38
1,2,3,4,6,7,8,9 OCN	4.32	2,9 Dibromoanthracene	1.16
HeptaCNs	5.05		

References: Wells and Echarri [7,93].

chemicals so that either saponification or concentrated sulphuric acid treatment can be used to degrade the lipids.

4.8.1. Saponification

Lipids can be saponified by heating the extract in a small volume of solvent with 20% ethanolic potassium hydroxide at ca. 70°C for 30 min. Van de Valk and Dao [94] found

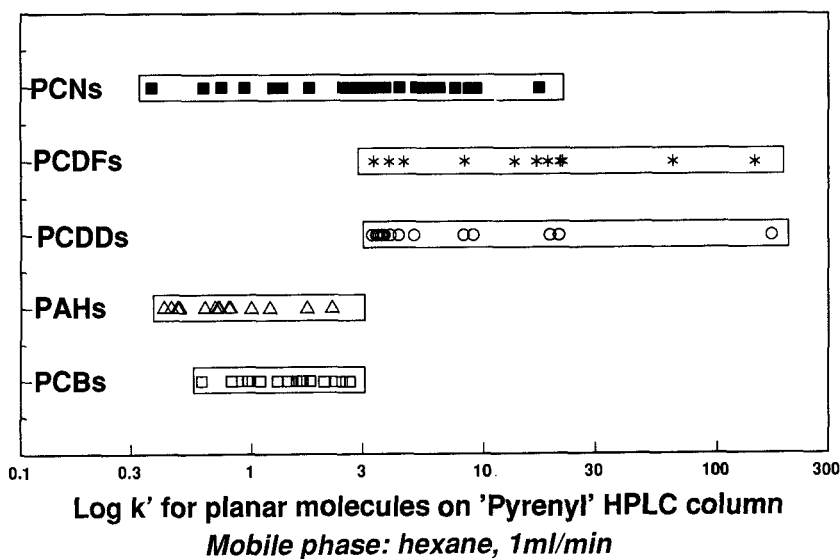


Fig. 4.8. Plot of the log k' capacity factors for polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzo-furans (PCDFs), PCNs, PAHs and chlorobiphenyls obtained on the pyrenyl-silica "PYE" HPLC column 150 × 0.46 mm i.d. using hexane as relevant 1 ml min⁻¹.

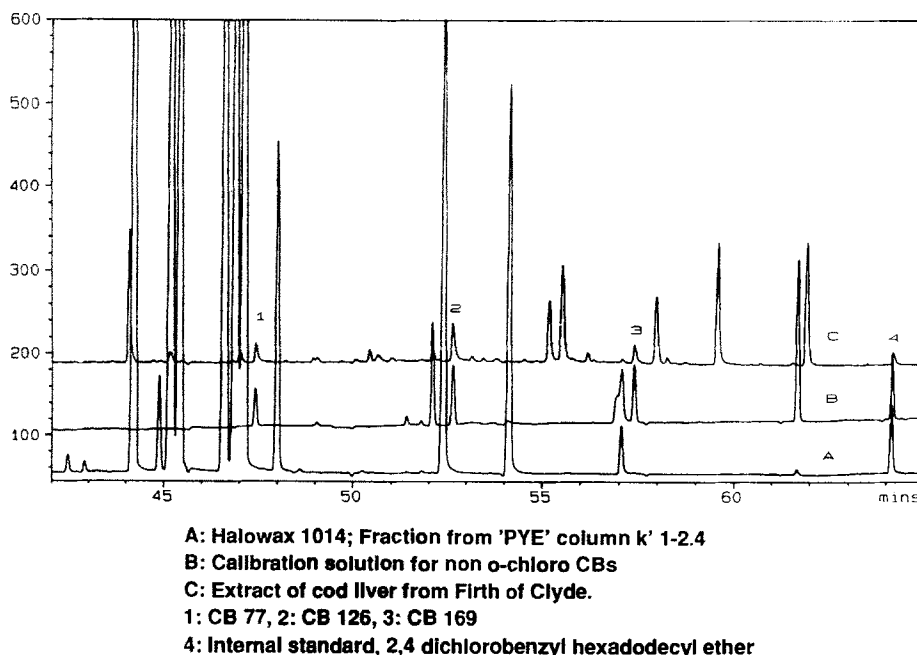


Fig. 4.9. Chromatogram of fraction III from HPLC pyrenyl column containing pentachloro naphthalenes and non-*ortho* chloro CBs showing the separation on CPSil 8 50 × 0.22 mm i.d., film thickness 0.22 μ m.

that CB 180 was partially degraded during the saponification of sewage sludge when temperatures were above 70°C for more than 30 min. However, when a standard CB mixture in hexane was treated in the same way there was no measurable degradation.

Wells and Echarri [95] initially used CB 209 as a recovery standard, but were only able to obtain ca. 50% from the saponification mixture. They repeated the saponification experiment with a fish oil spiked with a mixture of 51 CBs (National Research Council of Canada CLB standard) and found that CBs 170, 194, 195, 201, 205, 206, 207 and 209 were all hydrolysed with CB 170 degrading by ca. 10% over 90 min to CB 209 which was almost completely eliminated in 30 min (Fig. 4.10). All other CBs, including CB 180 in the CLB mixture were not affected by the reaction at temperatures below 70°C.

This hydrolytic degradation only affects the more chlorinated CBs, but could have implications for recovery experiments where CB 189 is used as a surrogate standard.

4.8.2. Sulphuric acid

The main alternative, destructive clean-up method to saponification is oxidative dehydration with concentrated sulphuric acid adsorbed onto silica gel. The degradation primarily removes lipids and wax esters, but many other co-extractants are also removed at the same time [6,7,95]. The main advantage of this technique is that it is fast, efficient and can remove large quantities of lipid (20 g or more). A column of 50 g of silica/sulphuric

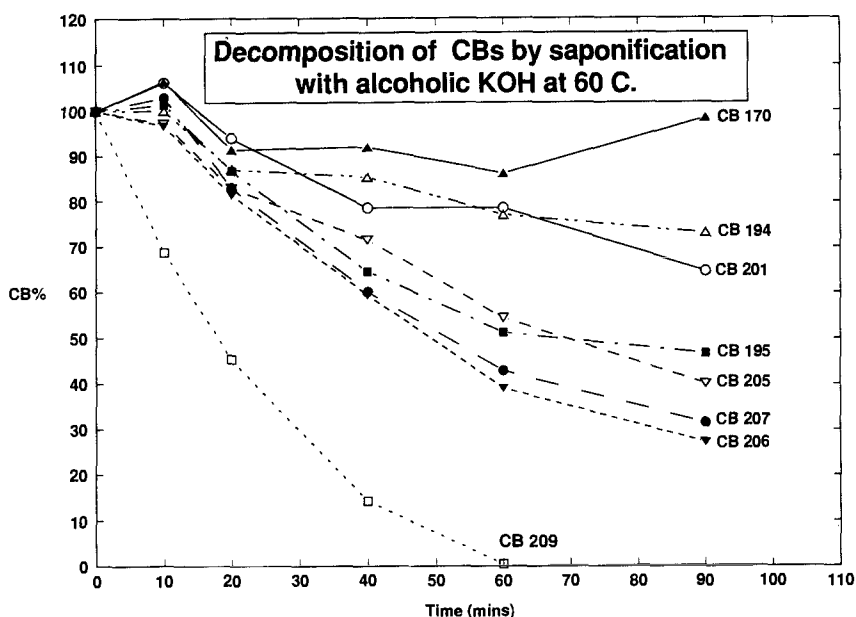


Fig. 4.10. Saponification of highly chlorinated CBs at 60°C with 20% ethanolic potassium hydroxide for different reaction times. Normally 30 min is sufficient to remove all lipids, but this does affect the higher chlorinated CBs.

acid can remove 10 g of lipid from an extract. Wells and Echarri [95] found that when removing such large quantities of fat, the microfine carbon formed tended to retain a small percentage of the CBs (ca. 2–3%) on the column. There was no difference in retention between the planar and non-planar CBs. The volume required to elute the CBs can be reduced by using dichloroethane in place of *n*-hexane.¹

4.8.3. Gel permeation (GPC)

GPC has also been used to remove large quantities from the extracts. The columns normally used are approximately 300–400 × 25 mm i.d. and packed with Biobeads SX3 200–400 mesh. Up to 500 mg of extractable lipid can be separated on these columns in a single injection. The two main advantages of this technique are that the method can be fully automated and that other contaminants such as more sensitive polar pesticides can be separated from the fat without being destroyed [96]. Tuinstra et al. [62] has used this technique for the removal of animal fat in the determination of planar CBs, and Haglund

¹**SAFETY:** Extreme care should be exercise when handling the silica/sulphuric acid powder. The silica powder which may become airborne with handling large quantities, is, in effect, microfine concentrated acid. The material should always be handled in a fully operational fume cupboard and a filter mask should be worn.

et al. [92], Haglund [97] and Jansson et al. [6] had a very similar system to separate the CBs in extracts of reindeer tissue, fish liver and seal blubber.

4.8.4. Dialysis

Polyethylene (PE) film of pore size ca. $50\mu\text{m}$ can be used to dialyse the organic extract to isolate the CBs from the fat. Around 10 g of fat in 15–20 ml pentane is placed in a cut section of pre-washed, PE “lay flat” tubing overnight in a beaker of pentane will dialyse around 95% or more of the CBs into the surrounding solvent (Fig. 4.11). The PE has a molecular cut-off of around 500 Da and acts as a static size exclusion membrane. This method is simple and effective in operation [98].

4.8.5. Adsorption columns

The use of adsorption chromatography for clean-up of lipid samples is well established. Alumina, silica and florisil [75–77] have been used in different mesh sizes, levels of activity and column sizes to separate the CBs from co-extracted materials and in group separation schemes to isolate the CBs from OCPs and other trace organics. The absorbents have been used separately and in combination to reduce the sample handling and analysis time. Each column system must be fully validated, not only for the particular physical configuration selected, but also for each new batch of adsorbent. Although 12–24 samples can be cleaned-up simultaneously, the technique is highly labour intensive and should be eventually superseded by an automatic “on-line” system. Alumina impreg-

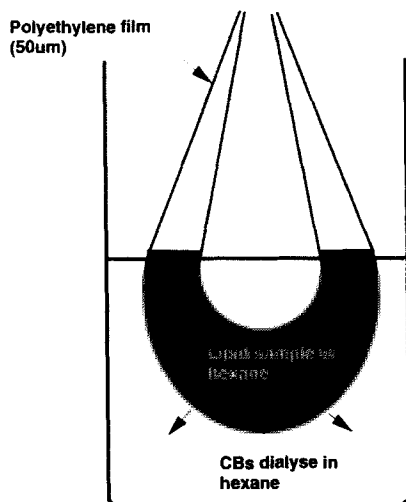


Fig. 4.11. Dialysis of small organic contaminants, including CBs, as a method of separation from high molecular weight lipids, using polyethylene film. The extract of CBs and lipids are dissolved in *n*-hexane.

nated with 20% potassium hydroxide has been used effectively for the clean-up of mineral oil and waste oil containing CBs [99].

Extracts from plant material and some types of organically rich sediments can give particular problems from negative peaks in the GC from aryl and alkyl hydrocarbons and from oxy and nitro heteroaromatics. In addition to the LC methods of clean-up, HPLC has been used to selectively remove these interferences and to isolate organochlorine compounds other than CBs. Greinval et al. [100] have used a 5 μ m 300 \times 3.9 mm μ -Bondapak aminopropyl silica column with hexane as an eluant to successfully separate PAH [101] and PCBs from chloroparaffin, polychlorocamphenes (PCC) and organochlorine pesticide (OCPs) in addition to other extraneous material.

4.9. EXTRACTION

Extraction and recovery of organochlorines, including CBs from soil, sediment, animals and plant tissue are covered in more detail elsewhere [96]. A schematic overview of the methods of analysis for CBs is given in Fig. 4.12 showing the various extraction, clean-up and separation techniques that have been used to prepare environmental samples for the measurement of CBs by ECD or MS. The main extraction methods used are Soxhlet extraction [7,64,126], blending [6,102,103,104] and more recently supercritical fluid extraction [105–111]. Most methods of isolating the CBs from their environmental matrix are now well established and the optimized methods can be validated with certified reference materials and surrogate recovery standards.

4.10. CHLOROBIPHENYL METABOLITES

Normally, metabolism of xenobiotics occurs as part of the detoxification process to form a more soluble derivative such as a hydroxy or poly-hydroxy product, which can be conjugated as a sulphate or glucuronide and excreted from the body [112].

In the case of CB metabolism, most of the lower chlorinated (di- and tri-chloro) congeners and a number of the more highly chlorinated CBs are metabolized in both aquatic and terrestrial mammals. However, while some of the CB metabolites are excreted as soluble conjugates, there is also a significant redistribution and accumulation of these metabolites in different tissues.

Following the initial reports of the presence of methyl sulphone CBs in Baltic seal in 1976 [2], work has been undertaken on the biotransformation of CBs to the methylsulphone metabolites [113,114].

The main group of CB metabolites that have been identified are the hydroxy, the methyl sulphoxide and methyl sulphone derivatives. The formation of the hydroxylated metabolites proceed via an arene oxide intermediate [115]. The methyl sulphone CBs (MeSO₂-CBs) are formed as a microbial metabolic transformation of the mercapturic acid pathway via a methyl sulphoxide intermediate [113,116,117]. Brandt and Bergmann [117] found that these methyl sulphones had a specific high binding affinity for soft tissues such as lung, kidney, brain and foetal tissue. Haragachi et al. [118,119] determined

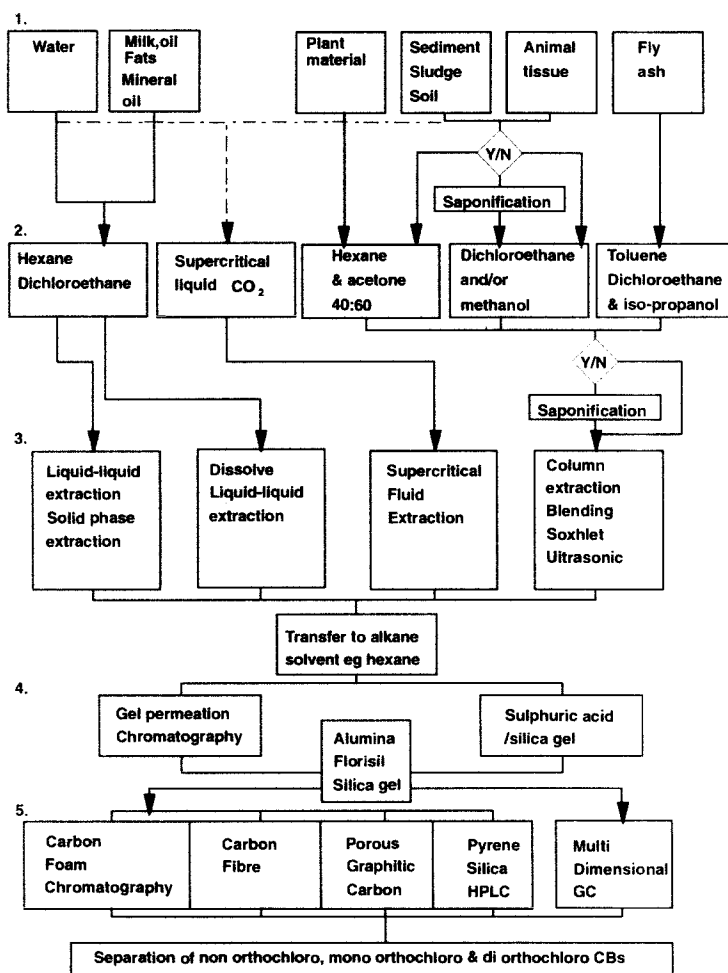


Fig. 4.12. Schematic flow diagram for the determination of CBs, including non-*ortho* and mono-*ortho* chloro congeners in environmental matrices.

hydroxymethyl sulphone metabolites of CBs in Yusho patients and methyl sulphones and sulphoxides in whale and fish.

Since these methyl sulphone and sulphoxide CB metabolites are present in relatively small concentrations, a relatively rigorous separation and concentration scheme is essential to isolate these compounds from the metabolites of other chlorinated contaminants and from the unmetabolized compounds.

The identification of methyl sulphones, sulphoxides and hydroxides have been limited by the number of the single pure compounds which have been synthesized and that are available. Most of the compounds have been synthesized in those laboratories where these studies have been made [119,120].

Non-polar or semi-polar columns (e.g. OV 101) that are thermally stable give good chromatographs and should be used since the methyl sulphone derivatives are less volatile than the CB precursors and will elute at a higher temperature. An HT-5 type, 12-dicarba-closo-dodecaborane polydimethylsiloxane (SGE) is even more suited to this application, and more tolerant of the higher temperatures.

Haraguchi et al. [118,119] used a programme from 200 to 270°C at 8° min⁻¹ with an OV 101 50 m × 0.25 mm i.d. column into a JEOL JMS DX300 MS to separate and identify hydroxymethyl sulphone CBs in samples taken from Yusho patients. The same group also used a 30 m × 0.2 mm fused silica Dexil 410 capillary column programmed at 180–270°C at 4° min⁻¹ into the same MS to study the methyl sulphone metabolites. A summary of the some recent studies on CB metabolites is given in Table 4.8.

Brandt and Bergmann [117] determined the deposition of 28 CBs and their metabolites in the different tissues of mice, rat and quail. There was a predominance of some specific CBs which were metabolized and accumulated in the lung tissue of the mice (Table 4.8). A number of CBs which are known to be metabolized in mammalian species formed the 4-MeSO₂-CB derivative. These were CBs 31, 49, 64, 70, 101 and 52. The CB 99 and CB 153 were not metabolized, although they both accumulated in the lung. These CBs are two of the limited number of congeners that remain unmetabolized in polar bear, *Ursus maritimus* [15]. The other CBs that were administered were CB 3, 8, 40, 42, 66, 47, 77, 123, 120, 168, 140, 180 and 196; none of which formed detectable levels of methyl sulphone or hydroxy derivatives in the tissues studied. Some CBs formed the hydroxy metabolites, e.g. CB 31, 3-OH 2,5,4'-trichlorobiphenyl and CB 77 2-OH 3,4,3',4'-tetrachlorobiphenyl and these accumulate in foetal soft tissue and uterine fluid [121]. The studies on CB metabolites thus far have indicated that the main metabolites are formed as an adduct rather than as a substitution of the chloro atom. The original CB substitution pattern, therefore remains intact. Some CBs are metabolized and excreted, probably as the hydroxy CB while others form methoxy sulphones and are firmly bound to soft tissue. What is clear from these studies is that the absence of the parent congener in mammalian tissue does not imply that the compounds have been excreted. In many cases, there is a redistribution of the metabolite to other target tissues such as the lung, kidney and foetal tissue. The metabolic patterns and tissue patterns are highly congener specific and until sufficient numbers of the individual metabolites are more widely available, it may be difficult to fully elucidate the biological pathways of these compounds.

The methyl sulphone and sulfoxide metabolites were extracted from the tissue by blending with dichloromethane and the extract was cleaned-up using GPC with Biobeads SX3 200–400 mesh. The sulphone fraction (120–150 ml) was eluted with dichloromethane/cyclohexane (1:1). At this stage in the clean-up, the bulk of the lipids were removed. This fraction was further chromatographed on basic alumina with 8% dichloromethane in *n*-hexane to remove the unmetabolized CBs, and most of the other related chlorinated compounds. The methyl sulfoxide and sulphone CBs were eluted with dichloromethane and partitioned in acetonitrile. The sample was passed through a C₁₈ Sepak cartridge and first eluted with acetonitrile/water (1:3) to remove the chloroparaffin metabolites. The ratio was then changed to 3:1 to elute the CB metabolites. After a sulphuric acid/*n*-hexane partition, the sample was further fractionated on a Bondapak-CN HPLC column

TABLE 4.8

CB METABOLITES AND THEIR PRECURSORS IN BIOLOGICAL TISSUE

Species	Tissue	Metabolite	Precursor	Reference
Polar bear	Liver	3-MeSO ₂ 2,5,2',4'	49	Kieroki et al. [123]
		4-MeSO ₂ 2,5,2',4'	49	
		3-MeSO ₂ 2,5,2',4',5'	101	
		4-MeSO ₂ 2,5,2',4',5'	101	
		3-MeSO ₂ 2,5,2',3',4'	87	
		4-MeSO ₂ 2,5,2',3',4'	87	
		4-MeSO ₂ 2,5,2',3',5',6'	151	
		4-MeSO ₂ 2,3,6,2',4',5'	149	
Mink and otter	Adipose tissue	3-MeSO ₂ 2,5,2',4',5'	101	Bergmann et al. [120]
		4-MeSO ₂ 2,5,2',4',5'	101	
		3-MeSO ₂ 2,3,6,2',4',5'	149	
	Thyroid	5-OH 3,4,3',4'	77	Willemssen et al. [124]
		4-OH 2,3,2',3',4'	82	
		4-OH 2,3,3',4'	56	
Mice, rat and quail	Lung	3-OH 3,3',4'	35	Brandt and Bergmann [117]
		4-MeSO ₂ 2,5,4'	31	
		3-MeSO ₂ 2,4,2',5'	49	
		4-MeSO ₂ 2,3,6,4'	64	
		3-MeSO ₂ 2,5,3',4'	70	
		4-MeSO ₂ 2,4,5,2',5'	101	
		Parent compound	99	
		Parent compound	153	
Mouse	Uterine fluids	3-MeSO ₂ 2,5,2',5'	52	Brandt and Bergmann [117]
		3-OH 2,5,4'	31	
	Foetal soft tissue	3-MeSO ₂ 2,5,4'	31	
		2-OH 3,4,3',4'	77	
Yusho patient	Lung and liver	MeSO ₂ 3,4,3',4'	77	Haraguchi et al. [119]
		6-MeSO ₂ 2,3'	6	
		5-MeSO ₂ 2,2'	4	
		6-MeSO ₂ 3,3'	11	
		5-MeSO ₂ 4,2'	8	
		5-MeSO ₂ 2,4'	8	
		4-MeSO ₂ 2,3'	6	
		5-MeSO ₂ 4,3'	13	
	Lung	5-MeSO ₂ 4,4'	15	Haraguchi et al. [118]
		4-MeSO ₂ 2,5,2',4'		
		Tri, tetra, penta MeSO ₂		
		4-MeSO ₂ 5-OH 2,2',3',4',5'		
		5-MeSO ₂ 6-OH 2,3,2',5'		

(100 mm × 8 mm i.d.) with a linear gradient 5–50% dichloromethane in *n*-hexane to separate the methyl sulfoxide CBs from the methyl sulphone CBs [119].

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Chapter 5

Official methods of analysis of priority pesticides in water using gas chromatographic techniques

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5.1. PRIORITY LISTS OF PESTICIDES

Several hundreds of pesticides of diverse chemical nature are widely used in the United States and Europe for agricultural and non-agricultural purposes. Some are substitutes for organochlorines, which were banned after evidence appeared of their toxicity, persistence and bioaccumulation in environmental matrices. According to a report published by the US EPA, a total of 500 000 tons of pesticides was used in 1985 [1]. Pesticide consumption in European countries such as United Kingdom was of the order of 14 000 tons per year during the 1980–1983 period [2]. As far as specific pesticides are concerned, worldwide consumption of malathion and atrazine in 1980 amounted to 24 000 and 90 000 tons, respectively [3,4]. In the Mediterranean countries, 2100 tons of malathion (active ingredient) were sprayed during the same period compared to 9700 tons in Asia [3].

A recent report published by the Commission of the European Communities indicated the total turnover of the major pesticides used in Denmark, France, Germany, United Kingdom, Greece, Holland, Italy, Spain and Sweden. The same report contained non-agricultural uses [5]. Atrazine, one of the herbicides most widely used in the United States and European countries over the last 30 years, is employed for pre- and post-emergence weed control of corn, wheat, barley and sorghum, and on railway and roadside verges. In this regard, in England and Wales alone, the non-agricultural use of this herbicide repre-

sented 140 tons of active ingredient whereas France accounted for 43 tons during 1989 [5]. Not surprisingly, it has been detected in ground- and surface-waters through the world (e.g. in some US ground-waters at concentrations in the range 0.1–3 $\mu\text{g/l}$ [1], as well as in ground-waters from different European countries [5] and in various estuarine areas such as the river Rhône in France [6] and in the Ebro delta in Spain [7]. An example of the contamination level by herbicides in the Ebro delta area (Tarragona) of Spain is shown in Fig. 5.1. The two charts in this figure show the different contamination levels of the river and the canals. A higher level of pollution (ca. 10 times higher) is noticed in the canals, owing to their proximity to the fields, where pesticides are applied, and to their low water-flow compared to the Ebro river.

5.1.1. European lists of pesticides

Owing to the environmental impact of pesticides, several priority lists, also called “red-” and /or “black lists”, have been published to help protect the quality of drinking- and surface-water. Table 5.1 shows the different pesticides listed in the 76/464/EC Directive (the so-called “black list”) [8]. Following the three general parameters (toxicity, persistence and input) for selecting the priority list of pollutants [9] in the United Kingdom, a “red-list” of substances that include several pesticides, most of them common to the EC list, was established [9]. For preventing the contamination of ground- and drinking water by pesticides in Europe, a priority list, which considers pesticides used in quantities over 50 tons per annum (and over 500 are underlined) and their capacities as probable or transient leachers, was recently published [5]. This is given in Table 5.2. There are a few other pesticides, such as demeton-S-methyl, fentin acetate, mancozeb, propineb, thiobencarb and zineb, that are being used in amounts over 50 tons per annum but for which there is, up to now, insufficient data for evaluating the probability of leaching. They are consequently not included in Table 5.2. In addition, glyphosate and thiram were not added to Table 5.2 because large differences in the ground-water ubiquity score (GUS) index were found. Such an index is a measure of the leaching capacity of the pesticide through the soil [5]. Another important remark on the different pesticides reported in Table 5.2 is that, although no transformation products (TPs) are included, a report published by the Commission of the European Communities [5] indicated that there is much interest in the analytical determination of such TPs for triazine, organophosphorus, carbamate and chlorinated phenoxy acid herbicides. In this respect, although the EC Directive on the quality of water intended for human consumption sets a maximum admissible concentration (MAC) of 0.1 $\mu\text{g/l}$, for individual pesticides and related products and 0.5 $\mu\text{g/l}$ for total pesticides, it is not clear what can be suggested as “related products”. It has been indicated that these “related products” refer to TPs that are toxic, which in the context of ground-water contamination could be interpreted as exceeding a water quality standard derived from toxicological considerations [5]. In this respect, it is clear that some specific TPs, such as fenitrooxon (from fenitrothion) and 1-naphthol (from carbaryl), are more toxic to aquatic organisms than are the parent compounds. The same applies to ethylene thiourea (ETU), which is a well known TP of maneb and related pesticides, and is more toxic than the parent pesticides [5].

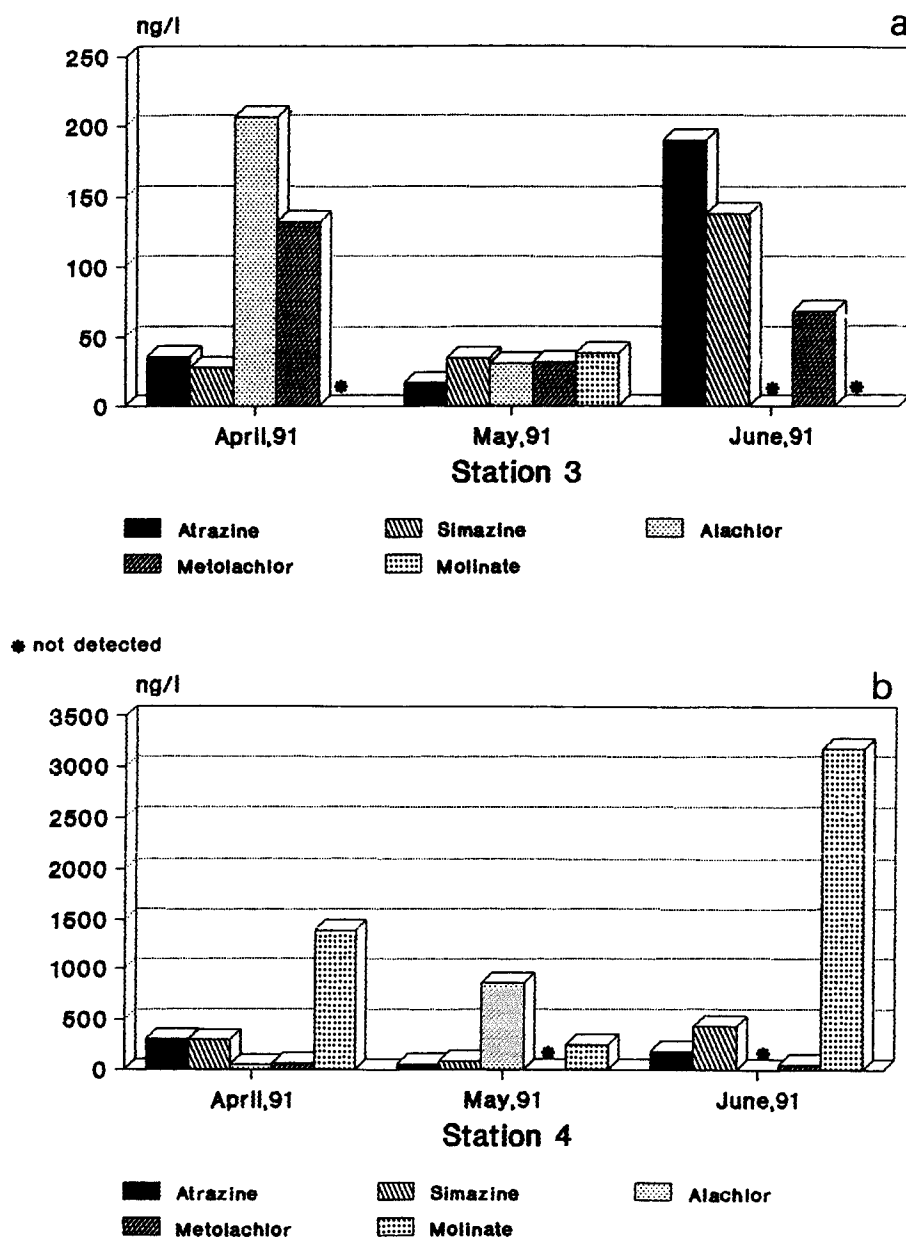


Fig. 5.1. Environmental levels of herbicides at two stations located (a) on the Ebro River and (b) on the Ebro delta drainage canals (Tarragona, Spain) obtained during a pilot monitoring programme carried out during April–June, 1991. Concentration levels are indicated in nanograms per litre. Asterisks denote herbicides not detected. (Reproduced in part with permission from [7].)

TABLE 5.1

PESTICIDES LISTED IN 76/464/EC COUNCIL DIRECTIVE ON POLLUTION CAUSED BY CERTAIN DANGEROUS SUBSTANCES DISCHARGED INTO THE AQUATIC ENVIRONMENT OF THE COMMUNITY (BLACK LIST)

Aldrin	Disulfoton	Monolinuron
Atrazine	Endosulfan	Omethoate
Azinphos-ethyl	Endrin	Oxydemeton-methyl
Azinphos-methyl	Fenitrothion	Parathion-ethyl
Chlordane	Fenthion	Parathion-methyl
Coumaphos	Heptachlor	Phoxim
2,4-D	Hexachlorobenzene	Propanil
DDT	Linuron	Pyrazon
Demeton	Malathion	Simazine
Dichlorprop	MCPA	2,4,5-T
Dichlorvos	Mecoprop	Triazophos
Dieldrin	Metamidophos	Trichlorfon
Dimethoate	Mevinphos	Trifluralin

TABLE 5.2

PESTICIDES USED IN EUROPE IN AMOUNTS OVER 50 tons PER ANNUM WHICH WERE CLASSIFIED AS PROBABLE OR TRANSIENT LEACHERS. PESTICIDES USED IN AMOUNTS OVER 500 tons ARE UNDERLINED

<u>Alachlor</u>	Dinoseb	<u>Methabenzthiazuron</u>
Aldicarb	<u>Diuron</u>	Methiocarb
Amitrole	DNOC	Oxydemeton methyl
<u>Atrazine</u>	EPTC	Phenmedipham
<u>Benazolin</u>	Ethofumesate	<u>Prochloraz</u>
<u>Bentazone</u>	Ethoprophos	Propham
Bromofenoxim	Fenamiphos	Prometryn
<u>Carbaryl</u>	Fluoroxypyr	<u>Propiconazole</u>
Carbendazim	Iprodione	Propyzamide
<u>Carbetamide</u>	<u>Isoproturon</u>	<u>Pyrethrin</u>
<u>Chloridazon</u>	Linuron	Simazine
Chlorpyrifos	<u>Maneb</u>	Terbutylazine
<u>Chlortoluron</u>	<u>MCPA</u>	Terbutryn
Cyanazine	<u>MCPP</u>	Triademinol
<u>2,4-D</u>	Metamitron	Trichlorfon
Dalapon	<u>Metazachlor</u>	<u>Trichloroacetic acid</u>
Diazinon	<u>Metham-sodium</u>	Vinclozolin
Dichlobenil	<u>Metolachlor</u>	Ziram
<u>Dimethoate</u>		

5.1.2. Mediterranean countries list

Following considerations based on usage information, physico-chemical properties and persistency, a priority list of herbicides was established for the Mediterranean countries France, Italy, Greece and Spain. The list, which is given in Table 5.3, considers selected herbicides that can cause contamination of estuarine and coastal environments. The selection of pollutants has been based on the availability of usage data and the consideration of half-lives so that pesticides that do not exceed a total of 10 tons after 90 days of application have been discarded [10]. Note that some of these pesticides are common to Table 5.2. In this respect, we should emphasize that pesticides in drinking water from groundwaters are affected in a different way from pesticides that reach estuarine waters. The transport of pesticides from river-waters to estuarine areas and coastal environments will be dependent on several parameters, for example on how they are absorbed into the suspended particulates and how they are affected by the higher salinity and pH. An example of such a type of contamination is provided by the Rhône estuary in the Camargue region, and is indicated in Fig. 5.2. It shows the total-ion-current chromatogram, obtained after extraction of 5 l of river-water extract with dichloromethane, of the dissolved (D) and particulate (P) organic matter. The levels of the different chlorotriazine herbicides varied from 1 to 17 ng/l in the dissolved phase, whereas in the particulate matter the levels found were below 1 ng/l [6]. This indicates that, for this particular group of herbicides, the transport from river-water to the sea occurs mainly in the dissolved phase.

5.1.3. National pesticide survey list

It is estimated that ground-water is the source of drinking water for 90% of rural households and three-quarters of US cities. In total, more than one-half of US citizens rely

TABLE 5.3

HERBICIDES OF POTENTIAL CONCERN IN THE MEDITERRANEAN REGION

Alachlor	EPTC	Molinate
Amitrole	Ethalfuralin	Napropamide
Atrazine	Ethofumesate	Neburon
Bentazone	Flamprop-M-isopropyl	Paraquat
Bromoxynil	Glyphosate	Pendimethalin
Butylate	Isoproturon	Phenmediphan
Carbetamide	Linuron	Prometryn
Chlortoluron	MCPA	Simazine
2,4-D	Mecoprop	Trichloroacetic acid
Di-allate	Metamitron	Terbumeton
Dichlobenil	Metazachlor	Terbutylazine
Dichlofop-methyl	Metabenzthiazuron	Terbutryn
Dinoterb	Metobromuron	Tri-allate
Diquat	Metolachlor	Trifluralin
Diuron	Metoxuron	
DNOC	Metribuzin	

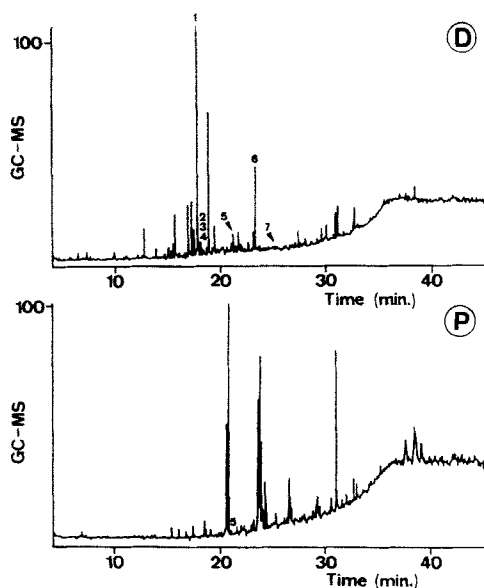


Fig. 5.2. Total ion current GC-MS of dissolved (D) and particulate (P) matter of river-water extract from one of the stations located at the Rhône river estuary. Sampling was carried out during November 1990. Extraction of 5 l of river water sample was carried out using dichloromethane, see ref. 6. Compounds identified corresponded to: (1) tributylphosphate; (2) de-ethylatrazine; (3) simazine; (4) de-isopropylatrazine; (5) atrazine; (6) caffeine; (7) propanil. Concentration levels expressed in nanograms per litre for de-ethylatrazine, simazine, de-isopropylatrazine, atrazine and propanil were 4, 10, 3, 17 and 2, respectively. A capillary GC DB-1701 column was used. (reproduced with permission from [6].)

on ground-water for their everyday needs. Because of the amount of information indicating the presence of pesticides in ground-water in the different US states [1], a joint research project between the Environmental Protection Agency (EPA)'s Office of Drinking Water (ODW) and the Office of Pesticide Programs (OPP) conducted a statistically based survey of pesticide contamination of drinking water wells. During the National Pesticide Survey (NPS), 1349 drinking water wells were sampled and analyzed for 101 pesticides, 25 pesticide TPs and nitrate, with a total of 127 analytes. The results of the NPS were released in November 1990 (Phase I) and January 1992 (Phase II) [11,12]. The selection of the different analytes was based on the use of at least 1 000 000 lb, of water solubility greater than 30 mg/l and hydrolysis half-life longer than 25 weeks. Pesticides and pesticide degradation products previously detected in ground-water and pesticides regulated under the Safe Drinking Water Act, were automatically included in this priority list [13]. The compounds were grouped according to their analysis and so seven methods were used that covered all the 126 analytes: they are indicated in Table 5.4 [14]. From the pesticides listed in Table 5.4, the stability was checked for a total of 147 analytes, 121 being stable during at least 14 days and stored in well-water at 4°C. Among the 26 unstable pesticides, which showed a loss of 100% after storing in the conditions mentioned above, were many organophosphorus pesticides such as azinphos-methyl, disulfoton,

fenitrothion, fenthion, malathion and parathion-methyl. One might mention that, although these organophosphorus pesticides are included in the priority list of compounds of the EC (see Table 5.1), their proved degradation under biologically inhibited conditions when stored at 4°C for 14 days in well-water indicates that they are not so harmful, and their incorporation in a priority list is doubtful. Other pesticides, such as ETU and heptachlor, had suffered slight degradation (between 15 and 22%) under identical storage conditions, whereas the sample extract generally remained stable [14].

The list indicated in Table 5.4 is the most complete list so far for conducting a monitoring programme on pesticides. In the last few years, from 1990, early-warning systems have been developed in Europe for the on-line screening and liquid-chromatographic detection of 50 polar pesticides and other pollutants in Rhine river-water. This project involves the Rhine basin, with research groups from Switzerland, Germany and the Netherlands. The first results on the analytical method development have been published recently [15,16]. They consider only the analytical development stage. One of the main differences between US and European regulations on pesticide programmes is that in Europe, each country is using its own analytical method whereas in the United States, the EPA methods are widely implemented. The different criteria between European governmental laboratories, which prefer to use conventional liquid-liquid extraction (LLE) procedures, and research or well-established laboratories, that prefer to use solid-phase extraction (SPE) techniques, means that within Europe, there is disagreement about the analysis of pesticides in water. Consequently, it is rather difficult to implement the NPS monitoring programme within the different countries, and intercomparisons and validation of the results are missing. This is one of the major problems within the EC, since an agency similar to the EPA does not exist in Europe, even though the Council of Ministers agreed to create the European Environmental Agency in 1990.

Some general comments can be made about the different priority lists presented in Tables 5.1–5.4. Although in some cases there is agreement on which priority pesticides to monitor, such as atrazine, 2,4-D, linuron and dimethoate, which correspond to different chemical groups, in other cases there is complete disagreement. This is the case, for example, with the carbamates, which have relatively high importance in the US monitoring programmes (see Table 5.4). The EPA has developed an excellent method for analysis of these pesticides in water at very low limits of detection (LOD). In contrast, in Europe, in the first black list of pesticides there were no carbamates at all (see Table 5.1). As they were not indicated in the first list of dangerous substances (Table 5.1) in Europe, no tradition of monitoring carbamates has been established, although its use has been reported in several countries, such as The Netherlands, Spain, United Kingdom and Italy. Furthermore, the EPA official method for monitoring carbamate pesticides (Method 531.1) has been seldom used in Europe, although it is a highly sensitive and robust method. Another aspect to consider should be the leachability of these carbamates through ground- and well-waters and they have been studied in this sense in the different well-waters of the USA through the NPS. In Europe, where the same sources are also important for drinking water, no planning in this sense has been undertaken. The percentage of ground-water used for drinking water purposes in Europe is close to 100% for Denmark, and has values of 85% for Italy, Germany, France and the United Kingdom, whereas in Spain it is in the region of 30%. A last remark on the different priority lists is that the NPS list (Table 5.4) is the only one that specifically mentions the TPs of

TABLE 5.4

PESTICIDES AND TPS INCLUDED IN THE US NATIONAL PESTICIDE SURVEY

Method 1 (EPA 507)

Alachlor	EPTC	Prometon
Ametryn	Ethoprop	Prometryn
Ametraton	Fenamiphos	Pronamide
Atrazine	Fenamirol	Propazine
Bromacil	Fluridone	Simazine
Butachlor	Hexazinone	Simetryn
Butylate	Merphos	Stirofos
Carboxin	Methyl paraoxon	(tetrachlorvinphos)
Chlorpropham	Metolachlor	Tebuthiuron
Cycloate	Metribuzin	Terbacil
Diazinon	Mevinphos	Terbufos
Dichlorvos	MGK 264	Terbutryn
Diphenamid	Molinate	Triademefon
Disulfoton	Napropamide	Tricyclazole
Disulfoton sulfone	Norflurazon	Vernolate
Disulfoton sulfoxide	Perbulate	

Method 2 (EPA 508)

Aldrin	Endosulfan I	Heptachlor
α -Chlordane	Endosulfan II	Heptachlor-epoxide
γ -Chlordane	Endosulfan sulfate	Hexachlorobenzene
Chlorneb	Endrin	Metoxychlor
Chlorobenzilate	Endrin aldehyde	<i>cis</i> -Permethrin
Chlorothalonil	Etridiazole	<i>trans</i> -Permethrin
DCPA	α -HCH	Propachlor
4,4'-DDD	β -HCH	Trifluralin
4,4'-DDE	δ -HCH	
4,4'-DDT	γ -HCH	
Dieldrin		

Method 3 (EPA 515.1)

Acifluorfen	Dicamba	Picloram
2,4-DB	3,5-Dichlorobenzoic acid	2,4,5-T
Bentazone	Dichlorprop	2,4,5-TP
Chloramben	Dinoseb	
2,4-D	5-Hydroxy dicamba	
Dalapon	4-Nitrophenol	
DCPA acid metabolites	PCP	

Method 4

Atrazine dealkylated	Fluometuron	Propanil
Barban	3-Ketocarbafuran phenol	Propham
Carbofuran phenol	Linuron	Swep

TABLE 5.4 (continued)

Cyanazine	Metribuzin DA	
Diuron	Metribuzin DADK	
Fenamiphos sulfone	Neburon	
Fenamiphos sulfoxide	Pronamide metabolite	
Method 5 (EPA 531.1)		
Aldicarb	Carbaryl	Methomyl
Aldicarb sulfone	Carbofuran	Oxamyl
Aldicarb sulfoxide	3-Hydroxycarbofuran	
Baygon (Propoxur)	Methiocarb	
Method 6		
ETU		
Method 7 (EPA 504)		
EDB	<i>cis</i> -1,3-Dichloropropene	
DBCP	<i>trans</i> -1,3-Dichloropropene	
1,2-Dichloropropane		

pesticides. This is very remarkable because although in the EC regulations the importance of TPs of pesticides is indicated [5], there is no mention of specific TPs. This indication in the EC list is more vague, thus making it more difficult for laboratories currently involved in monitoring programmes to follow and select the different TPs. In addition, it should be mentioned that many of the TPs need specific methods of analysis, and are poorly recovered under conventional screening methods. So, in this sense, the efforts made through the NPS with specific methods of analysis, and the list of priority pesticides and TPs provided in Table 5.4 are of great importance. Indeed, once the peculiarities of each country have been considered, studies can be implemented worldwide.

5.1.4. Health advisory levels

As pointed out previously [9], in the selection of a priority list one relevant parameter to take into consideration is the toxicity of the compound. Such toxicity depends on the compound, and its concentration in water will affect humans or the different aquatic organisms in different ways. For drinking water, the Commission of the European Communities has fixed a level of $0.1 \mu\text{g/l}$ for individual pesticides and $0.5 \mu\text{g/l}$ for total pesticides. This is a very strict requirement and analytical methods still need to be developed for a variety of pesticides to comply with such Directives. The Office of Water of the US EPA has established drinking water regulations and health advisory levels for individual pesticides. A selection of the different health advisory levels, also called the Maximum Contaminant Level Goals by the EPA, are indicated in Table 5.5. Values in this table have been selected from refs. 12 and 17. Such levels are more sensible than the EC levels, which are fixed for all the individual pesticides without making any distinction between individual pesticides of different toxicity. As for the levels of the TPs, it can be interpreted that they should follow toxicity values [9]. The establishment of maximum concentration levels for any individual pesticide is of greatest importance for compliance

TABLE 5.5

HEALTH ADVISORY LEVELS ($\mu\text{g/l}$) FOR SELECTED PESTICIDES IN DRINKING WATER
(EPA OFFICE OF GROUND-WATER AND DRINKING WATER)

Compound	Health advisory level ($\mu\text{g/l}$)	Compound	Health advisory level ($\mu\text{g/l}$)
Alachlor	2	Diquat	20
Aldicarb	10	Endothall	100
Aldicarb sulfoxide	10	Endrin	2
Aldicarb sulfone	10	Glyphosate	700
Atrazine	3	Methomyl	200
Bromacil	80	Methyl parathion	2
Carbofuran	40	Metolachlor	10
Chlorthalonil	2	Oxamyl	200
Cyanazine	9	Picloram	500
2,4-D	70	Simazine	4
Dalapon	200	Trifluralin	2
Dinoseb	7		

with the different Directives. In the EC, the strict Directive has the disadvantage that some ubiquitous pesticides, such as atrazine, which are not so toxic for human consumption, are often found at levels higher than $0.1 \mu\text{g/l}$, and one can observe in the literature that some EC countries exceed the EC regulations. Indeed, the levels established in the EC Directive are somewhat arbitrary as they were not based on toxicological studies and so, in some cases, a certain permissiveness has been accepted. The advantage of such a restrictive regulation for pesticides in Europe is that analytical methods are being developed that can detect pesticides at levels between $0.02 \mu\text{g/l}$ and so quantify them at $0.1 \mu\text{g/l}$. In this respect, analytical methods of sufficient accuracy and sensitivity are needed. In addition to the lack of information on which specific pesticides to monitor, it will also be impossible to determine at this level of sensitivity all the pesticides approved for use within the EC. Thus, it is difficult to know which pesticides will need analytical methods for monitoring. One approach is to focus resources on those pesticides which are likely to reach water resources, are used in sufficient amounts and have a tendency to be persistent and mobile (see Table 5.2). In addition, the EC Drinking Water Directive sets a limit of $0.5 \mu\text{g/l}$ for total pesticides. It is difficult to carry out proper monitoring of such a parameter, particularly in relation to defining the required detection limits and accuracy, unless an arbitrary maximum number of total pesticides is assumed. In the most recent report of the EC [5], it was stated that analytical methods need a detection limit of $0.02 \mu\text{g/l}$ or less (ideally $0.01 \mu\text{g/l}$), and need to provide data of sufficient accuracy. The aim should be around 20% total error (random and systematic), to be compatible with the need of the Drinking Water Directive of the EC. The pesticides of high priority to the EC are listed in Table 5.2. It has also been recommended [5] that significant analytical results should be confirmed by an additional technique, preferably involving some form of mass

spectrometry, because of the likelihood of false positives with the commonly applied methods such as gas chromatography with electron capture or nitrogen-phosphorus detection (GC-NPD) or liquid chromatography (LC) with UV detection. For some difficult pesticides, including maneb, ziram and trichloroacetic acid in water, analytical methods should be developed in order to reach the LOD indicated in the EC regulations. In the United States, most of the EPA methods in use comply with the Health Guidance levels indicated in Table 5.5. The EC Directive which regulates drinking water mentions pesticides and related products. Such "related products" refers to TP's that are toxic, which in the context of ground-water contamination could be interpreted as exceeding a water quality standard derived from toxicological considerations [5].

5.2. GENERAL CONSIDERATIONS ABOUT ANALYTICAL METHODS

In this chapter, the development of methods of analysis and confirmation for most of the "conventional" organochlorinated (OC) pesticides will not be considered, because most of them have been withdrawn and substituted by organophosphorus compounds and carbamates. Most of the organochlorinated pesticides are indicated under Method 2 (see Table 5.4) with a few exceptions such as chlordane, chlorobenzilate, chlorothalonil, etridiazole, methoxychlor, *cis*- and *trans*-permethrin, propachlor and trifluralin, which cannot be considered as conventional OCs. In this chapter the term "modern pesticide" is used: (i) to indicate that conventional (mostly withdrawn) OCs are not discussed; (ii) "modern" is preferred to "polar", since a few pesticides currently being used, such as *cis*- and *trans*-permethrin, chlorpyrifos and trifluralin are rather non-polar pesticides, so the term "polar" seems inadequate, although much used in the literature; and (iii) "modern" has been used in recent years to announce an international Workshop on such pesticides and has been much accepted in the scientific community.

In the present chapter, we discuss mainly the official methods of analysis of pesticides in drinking water used within the US EPA-NPS and in the United Kingdom. Another restriction will be made to GC methods of analysis, since two other chapters of this book deal with coupled-column liquid chromatographic (LC) and LC-mass spectrometric (MS) methods for the determination of the more polar pesticides and several TP's.

References and examples are given to developed methods involving the possibilities of detection by GC combined with NPD, ECD or FPD. As stated previously, it is compulsory to use confirmation methods to avoid false positives. In this sense, the use of MS techniques and of two different GC columns of different polarity for confirmation of pesticides in water (the common EPA procedure) are emphasized. A few examples and criticisms of the different approaches are given, along with their main advantages and disadvantages.

One of the main questions to be asked at the beginning of each analysis of pesticides by a variety of chromatographic techniques is whether to use GC or LC. In some instances, the choice can be very clear and for sufficiently volatile compounds, such as most organochlorinated pesticides, some organonitrogen pesticides such as atrazine, or organophosphorus pesticides such as fenitrothion, GC will be preferred. The problem arises when thermally labile and/or polar pesticides need to be analyzed. The use of derivatization techniques and further GC analysis with a selective detector, generally allows good

LOD. Some authors prefer the use of LC techniques, without prior derivatization and this simplifies the method. Some specific cases of polar and thermally labile pesticides need careful attention in GC analysis. They may give chromatographic peaks in the GC traces which correspond not to the compound itself but to a degradation product, generally formed in the injection port. One of the problematic groups is the carbamates, which show proven thermal instability when analyzed under conventional GC conditions. It has been pointed out that some of them can be analyzed by GC with careful selection of the instrumental conditions of analysis [18]. In this sense, for the analysis of carbaryl and other carbamates, such as carbofuran, the use of cold on-column injection has been reported to give good recovery values after isolation from water samples [19]. From the same group of compounds, aldicarb sulfoxide and aldicarb sulfone have also been studied, and it has been shown that aldicarb, their parent herbicide, degrades at injection-port temperatures of 130°C and that longer GC capillary columns do not allow elution in a reasonable time, with the result that thermal degradation is observed [20]. Indeed, a previous US EPA method oxidized aldicarb to aldicarb sulfone, by treatment with peracetic acid and then the sulfone was thermally degraded in the injection port to produce the volatile species 2-methyl-2-(methylsulfonyl) propionitrile (EPA, 1981) [21]. It has also been recommended, if GC methods are still to be used, that one can overcome the problems of decomposition of carbamates (which give phenols and isocyanates) by performing a prior derivatization using appropriate agents such as acetic anhydride [22]. Similar considerations could be applied for oxamyl and benomyl.

In the case of phenylurea herbicides, linuron can be analyzed by GC [19, 23] with cold on-column injection, but certainly monuron and diuron are too thermally unstable and degrade in the GC [23]. To avoid these problems, derivatization with appropriate reagents, such as heptafluorobutyric anhydride [24] can solve the problem and allow GC to be applied.

However, it is difficult in some cases to make a choice between GC or LC techniques for the determination of pesticides. The present chapter focuses on different examples, showing the procedures of the official methods such as EPA, and other methods that are being developed. In many instances, the choice of one method over the others depends on the experience of the laboratory, which is related to its facilities and know-how on the analysis of pesticides in water.

5.3. EPA METHODS OF ANALYSIS

Two reports with revisions of methods for the determination of organic compounds in drinking water have recently been published [25,26]. Revision and comments to the different EPA methods for water have also been discussed in two recent papers. These recommend dropping some of the 600 series, especially, and revision and encouragement of the use of capillary columns in GC, the use of micro-extraction methods, and the increasing use of GC-MS methods [27,28]. In this sense, for example from the different methods for analyzing purgeable organics, it is recommended that the EPA method 524.2 be kept, and that all the others (524.1 and 624) be dropped [27].

The philosophy behind the EPA methods is clearly stated in their objectives, to develop and evaluate analytical methods for organic contaminants in water, to determine

the responses of the aquatic organisms to water quality, and to develop a quality assurance programme to support the achievement of data-quality objectives. The different EPA methods used for pesticide analysis in water can be divided into three groups: (i) those that use GC with a selective detector (ECD or NPD); (ii) those that use GC-MS; (iii) those that use LC. Numbering of the different methods uses the groups of pesticides as they are given in Table 5.4. It is necessary to indicate that most of the EPA methods for pesticides in water use LLE procedures, with the exception of Method 525.1, which uses SPE procedures with either C-18 cartridges or Empore extraction disks. Although in this chapter, it is not intended to discuss aspects of sample preparation, SPE is gaining in importance since it avoids emulsions, and the consumption and disposal of large volumes of toxic and flammable solvents [29].

General properties of most of the EPA methods are: (i) the acceptance of recoveries in the range of 70 up to 130%, with a maximum relative standard deviation of 30% each; (ii) sample collection, preservation and storage is carried out at 4°C, with the recommendation whether a sample should be analyzed within a few days of storage or can be kept for a maximum of 14 days in water [14]; (iii) the apparatus and equipment are described with safety considerations concerning the reagents, standards and consumable materials; (iv) use is made of two columns of different polarity, the so-called primary column and a second column, called the confirmation column; (v) instructions on how to proceed with blank samples, internal standards and surrogate solutions, interferences, calibrations, standardization and quality control. Since the use of surrogate and internal standards can lead to some confusion; definitions of both terms are given. An internal

TABLE 5.6

EPA METHOD 505: ANALYSIS OF ORGANOHALIDE PESTICIDES AND COMMERCIAL POLYCHLORINATED BIPHENYL (PCB) PRODUCTS IN WATER BY MICROEXTRACTION AND GC-ECD

Summary of the method	35 ml of sample are extracted with 2 ml of dichloromethane; 2 μ l are injected onto the GC-ECD; extraction and analysis time 30–50 min
GC columns used	
Primary column	0.32 mm i.d. \times 30 m long, fused silica capillary column with DB-1
Confirmatory columns	0.32 mm i.d. \times 30 m long fused silica capillary column with 1:1 mixed phase of dimethylsilicone and polyethylene glycol (Durawax-DX 3) or equivalent or 0.32 mm i.d. \times 25 m long 50:50 methyl-phenylsilicone (OV-17) or equivalent
Method detection limits (MDLS) only for the modern pesticides (μ g/l)	
Alachlor	0.075
Atrazine	2.4
Metoxychlor	0.96
Simazine	6.8

TABLE 5.7

EPA METHOD 507 (NPS METHOD 1, SEE TABLE 5.4): DETERMINATION OF NITROGEN- AND PHOSPHORUS-CONTAINING PESTICIDES IN WATER BY GC-NPD

Summary of the method	1 l of water is extracted with dichloromethane by shaking in a separatory funnel; the extract is isolated and concentrated to a volume of 5 ml after solvent substitution with methyl tertiary butyl ether (MTBE)				
GC columns used					
Primary column	0.25 mm i.d. × 30 m DB-5				
Confirmatory column	30 m long × 0.25 mm i.d., DB-1701				
Surrogate	1,3-Dimethyl-2-nitrobenzene				
Internal standard	Triphenyl phosphate				
Estimated detection limits (EDLS) (μg/l)					
Alachlor	0.4	EPTC	0.2	Perbulate	0.1
Ametryn	2.0	Ethoprop	0.2	Prometon	0.3
Ametraton	0.6	Fenamiphos	1.0	Prometryn	0.2
Atrazine	0.1	Fenamirol	0.4	Pronamide	0.8
Bromacil	2.5	Fluridone	3.8	Propazine	0.1
Butachlor	0.4	Hexazinone	0.8	Simazine	0.1
Butylate	0.1	Merphos	0.2	Simetryn	0.2
Carboxin	0.6	Methyl paraoxon	2.5	Stirofos	0.8
Chlorpropham	0.5	Metolachlor	0.7	Tebuthiuron	1.3
Cycloate	0.2	Metribuzin	0.1	Terbacil	4.5
Diazinon	0.2	Mevinphos	5.0	Terbufos	0.5
Dichlorvos	2.5	MGK 264	0.5	Terbutryn	0.2
Diphenamid	0.6	Molinate	0.1	Triademefon	0.6
Disulfoton	0.3	Napropamide	0.2	Tricyclazole	1.0
Disulfoton sulfone	3.8	Norflurazon	0.5	Vernolate	0.1
Disulfoton sulfoxide	0.4				

standard is added to measure the relative responses of other analytes and surrogates that are components of the solution. It is a requirement that the internal standard must be an analyte that is not a sample component. In contrast, a surrogate analyte is a compound which is extremely unlikely to be found in any sample but is added to a sample aliquot before extraction and is measured by the same procedures used to measure other sample components. The purpose of a surrogate is to monitor the method-performance with each sample. The use of a surrogate and an internal standard is not common in other official methods, since they generally use only an internal standard. A final general comment on the different EPA methods is that, although they sometimes use MDLs or EDL or LOD, they seem to indicate the same idea, but with a different terminology. It will be recommended that in the future such criteria should be unified and that similar terms should always be used to indicate the LOD of a method.

A summary of each method used for the determination of modern pesticides and their corresponding TPs is indicated in Tables 5.6–5.14. In Table 5.6, a micro-extraction

method using GC-ECD detection is described. This indicates that, although the water volume used is only 35 ml, the method detection limits (MDLs) are quite acceptable, considering the water extracted. Conventional analytical methods for organics in drinking water use volumes of 1 l, at least.

In Table 5.7, the EPA method corresponding to organonitrogen- and organophosphorus-containing pesticides is shown. In this case, special attention is paid to the storage of the water samples. As mentioned earlier [14], it has been shown in the recent NPS that 26 organophosphorus pesticides were unstable, with 100% loss when stored under the usual conditions at 4°C during 14 days. Among them, disulfuton sulfoxide, diazinon, fenitrothion, pronamide and terbufos need to be mentioned, since their analytical determinations should be carried out immediately after extraction. Other analytes, such as carboxin, EPTC, fluridone, metalochlor, napropamide, tebuthiuron and terbacil, exhibited recoveries of less than 60% after 14 days. For such compounds, it was also pointed out that the sample extracts, stored under identical conditions, exhibited stability during 28 days, but storage for only 14 days was recommended. Figures 5.3 and 5.4 show two examples of the use of LLE with dichloromethane for the determination of stirofos (also called tetra-chlorvinphos), an organophosphorus pesticide, and various organonitrogen pesticides, respectively, in the Ebro delta waters. By extracting 5 l of water, with a reduction of the final volume to 0.5 ml, a LOD down to 0.1 ng/l can be obtained, as observed in Fig. 5.4 [7].

The EPA method for OC pesticides is briefly described in Table 5.8. As for Table 5.6, only the modern pesticides are indicated. Careful attention should be paid to certain pesticides such as chlordhalonil, *cis*-permethrin, *trans*-permethrin and trifluralin since the preservation data are non-definitive, and therefore one is recommended to analyze the samples immediately. For the other modern pesticides, the samples are stable for 7 days

TABLE 5.8

EPA METHOD 508 (NPS METHOD 2, SEE TABLE 5.4): DETERMINATION OF CHLORINATED PESTICIDES IN GROUND WATER BY GC-ECD

Summary of the method	1 l of water is extracted with dichloromethane by shaking in a separatory funnel; the methylene chloride extract is isolated and concentrated to a volume of 5 ml after solvent substitution with MTBE				
GC columns used					
Primary column	0.25 mm i.d. × 30 m DB-5				
Confirmatory column	30 m long × 0.25 mm i.d. DB-1701				
Surrogate	4,4'-Dichlorobiphenyl (DCB)				
Internal standard	Pentachloronitrobenzene (PCNB)				
Estimated detection limits (EDLS) (µg/l) (only for the modern pesticides)					
Chlorneb	0.50	Etridiazole	0.02	Propachlor	0.50
Chlorobenzilate	5.00	Metoxychlor	0.05	Trifluralin	0.02
Chlorthalonil	0.02	<i>cis</i> -Permethrin	0.50		
DCPA	0.02	<i>trans</i> -Permethrin	0.50		

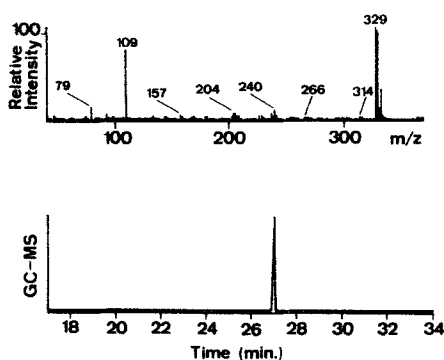


Fig 5.3. Spectrum and selected ion chromatograms in EI GC-MS of tetrachlorvinphos in water samples from the Ebro delta. Ions monitored corresponded to m/z 109 and 329 for tetrachlorvinphos (stirofos). Concentration level in the water: $5 \mu\text{g/l}$; 1 l of water was LLE with dichloromethane with a final volume of 0.5 ml. Injection volume: $1 \mu\text{l}$. A capillary GC FSQT RSL-300 column was used.

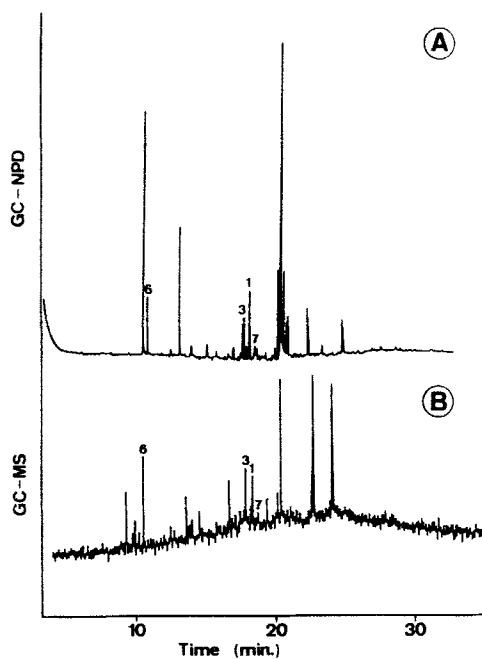


Fig. 5.4. (A) GC-NPD and (B) GC-MS of an extract of water sample from the Ebro delta containing (6) molinate ($0.050 \mu\text{g/l}$); (3) atrazine ($0.010 \mu\text{g/l}$); (1) simazine ($0.012 \mu\text{g/l}$); (7) alachlor ($0.005 \mu\text{g/l}$). A capillary GC DB-225 column was used; 4 l of estuarine water were LLE with dichloromethane and the final volume was 0.2 ml. Injection volume: $1 \mu\text{l}$. A capillary GC DB-225 column was used. (Reproduced with permission from [7].)

TABLE 5.9

EPA METHOD 515.1 (NPS METHOD 3, SEE TABLE 5.4): DETERMINATION OF CHLORINATED ACIDS IN GROUND WATER BY GC-ECD

Summary of the method	1 l of water is adjusted to pH 12 and shaken for 1 h to hydrolyze derivatives; extraneous inorganic material is removed by a solvent wash; the sample is acidified and the chlorinated acids are extracted with diethyl ether; the acids are converted to their methyl esters using diazomethane as derivatizing agent; excess of derivatizing agent is removed and the esters are determined by GC-ECD				
GC columns used					
Primary column	0.25 mm i.d. \times 30 m DB-5				
Confirmatory column	30 m long \times 0.25 mm i.d. DB-1701				
Surrogate	2,4,-Dichlorophenylacetic acid (DCAA)				
Internal standard	4,4'-Dibromooctafluorobiphenyl (DBOB)				
Estimated detection limits (EDLS) ($\mu\text{g/l}$)					
Acifluorfen	0.10	Dicamba	0.08	Picloram	0.14
3,5-Dichlorobenzoic acid	0.06	Dichlorprop	0.30	2,4,5-T	0.08
Bentazone	0.20	Dinoseb	0.20	2,4,5-TP	0.07
Chloramben	0.10	5-Hydroxy dicamba	0.04		
2,4-D	0.20	4-Nitrophenol	0.10		
Dalapon	1.30	PCP	0.08		
2,4-DB	0.80				
DCPA acid metabolites	0.02				

under refrigeration at 4°C. For organochlorinated pesticides, the table gives very low LOD values, in the range of 0.02–0.5 $\mu\text{g/l}$, with the exception of chlorobenzilate. Trifluralin can also be analyzed by GC-NPD with a low LOD [7]. Table 5.9 indicates a commonly used method for the determination of the acidic herbicides in water. These compounds are commonly used in Europe (e.g. bentazone, 2,4-D) and the United States (e.g. DCPA). The analytical method involves pH changes and a derivatization step with the formation of the methyl esters, followed by GC-electron capture (ECD) determination. The LOD are in the range of 0.04–0.8 $\mu\text{g/l}$, which is quite acceptable for this group of compounds.

The analyses of the more volatile compounds used as pesticides are indicated in Table 5.10. A purge-and-trap method with subsequent GC-MS determinations, and a micro-extraction method using *n*-hexane are described. In one case, the use of MS ensures unequivocal identification of the compounds and the use of a micro-extraction method avoids volatility problems to reach an acceptable estimated detection limit. In Table 5.11 a method for endothall is described involving derivatization and GC-ECD analysis. This pesticide method is considered “unusual” compared to other EPA methods. One of the most recent methods is described in Table 5.12 and is based on SPE techniques. A few EPA methods have been changed during the last few years, to incorporate SPE instead of LLE procedures. In this method, only one GC column is needed, since there is MS con-

TABLE 5.10

EPA METHOD 524.2 (NPS METHOD 7, SEE TABLE 5.4): MEASUREMENT OF PURGEABLE ORGANIC COMPOUNDS IN WATER BY CAPILLARY COLUMN GC-MS

Summary of the method	Volatile organic compounds and surrogates with low water solubility are extracted (purged) from the sample matrix by bubbling an inert gas through the water; purged samples are trapped in a tube containing suitable sorbent materials; when purging is completed, the sorbent tube is heated and backflushed with helium to desorb the sample components and analyzed by GC-MS
GC column used	60 m × 0.75 mm i.d.; Vocol glass wide-bore capillary with 1.5 µm film thickness or 30 m × 0.53 mm i.d. DB-624, with 3 µm film thickness or 30 × 0.32 mm i.d. DB-5, with 1 µm film thickness
Surrogates	4-Bromofluorobenzene and 1,2-dichlorobenzene- <i>d</i> ₄
Internal standard	Fluorobenzene

Method detection limit (µg/l) (using cryogenic trapping and narrow-bore capillary column)

1,2-Dibromoethane (EDB)	0.02
1,2-Dibromo-3-chloropropane (DBCP)	0.05
1,2-Dichloropropane	0.02
<i>cis</i> -1,3-Dichloropropene	n.d.
<i>trans</i> -1,3-Dichloropropene	n.d.

EPA METHOD 504 (NPS METHOD 7, SEE TABLE 5.4): 1,2-DIBROMOETHANE (EDB) AND 1,2-DIBROMO-3-CHLOROPROPANE (DBCP) IN WATER BY MICROEXTRACTION AND GC

Summary of the method	35 ml of sample are extracted with 2 ml of hexane; 2 µl of the extract are injected into a GC-ECD
GC columns used	
Primary column	0.32 mm i.d. × 30 m long fused silica capillary column with 1:1 mixed phase of dimethylsilicone and polyethylene glycol (Durawax-DX 3)
Alternative column	0.32 mm i.d. × 30 m long, fused silica capillary column with DB-1, or a wide-bore column of 0.53 mm i.d. × 30 m long with 2 µm film thickness of dimethyldiphenyl polysiloxane, bonded phase
Estimated detection limits (EDLS) (µg/l)	
EDB	0.01
DBCP	0.01

n.d. = not determined.

TABLE 5.11

EPA METHOD 548: DETERMINATION OF ENDOTHALL IN DRINKING WATER BY AQUEOUS DERIVATIZATION, LIQUID-SOLID EXTRACTION AND GC-ECD

Summary of the method	5 ml of water is placed in a Kuderna–Danish tube and the volume is reduced to less than 0.5 ml; the tube is charged with glacial acetic acid and sodium acetate followed by a solution of the derivatization reagent, pentafluorophenylhydrazine (PFPH). After heating at 150°C for 90 min, the derivative is extracted by a solid sorbent from the reaction solution, followed by elution with 5.0 ml of MTBE; the MTBE extract is analyzed by GC-ECD
GC columns used	Supelco SPB-5, 0.25 mm × 30 m; as a second column can be used a DB-1, 0.32 mm × 30 m or equivalent
Internal standard	Endosulfan I
Method detection limit (µg/l)	11.5

firmation by the fragment ions of each pesticide. In this method, special attention should be paid to possible sources of contamination of the cartridges or disks, which often contain phthalate esters, silicon compounds and other contaminants [30,31]. There are a few requirements for the MS such as: (i) scanning should be performed between 45 and 450 amu; (ii) the calibrant for the MS, bis (perfluorophenyl)phenylphosphine (decafluoro-

TABLE 5.12

EPA METHOD 525: DETERMINATION OF ORGANIC COMPOUNDS IN DRINKING WATER BY LIQUID-SOLID EXTRACTION AND CAPILLARY COLUMN GC-MS

Summary of the method	Organic compounds are extracted from a water sample by passing 1-l of sample through a cartridge or a disk containing a solid inorganic matrix coated with a chemically bonded C-18 organic phase; the organic compounds are eluted from the LSE cartridge or disk with a small quantity of dichloromethane and concentrated further by evaporating some of the solvent; final concentration of the extract is between 0.5 ml and 1 ml; an aliquot of 1–2 μ l is injected onto the GC-MS and compounds are identified by their retention times and mass spectra		
GC column used	A 30 m \times 0.25 mm i.d. capillary column coated with DB-5 or equivalent is recommended		
Surrogate	Perylene-D ₁₂		
Internal standards	Acenaphthene- D ₁₀ , phenanthrene D ₁₀ and chrysene D ₁₀		
Estimated detection limits (EDLS) (μ g/l) (only for modern pesticides)			
Alachlor	0.09	Metoxychlor	0.08
Atrazine	0.14	Simazine	0.12

TABLE 5.13

EPA METHOD 552: DETERMINATION OF HALOACETIC ACIDS IN DRINKING WATER BY LIQUID-LIQUID EXTRACTION, DERIVATIZATION AND GC-ECD

Summary of the method	100 ml of water is adjusted to pH 11.5 and extracted with MTBE to remove neutral and basic organic compounds; the aqueous sample is acidified to pH 0.5 and the acids are extracted into MTBE; after the extract is dried, the acids are converted to their methyl esters with diazomethane (DAM); excess of DAM is removed and the methyl esters are determined by GC-ECD
GC columns used	30 m \times 0.32 mm i.d. of DB-1701 and as a confirmatory column a 30 m \times 0.32 mm i.d. DB-210
Method detection limit ($\mu\text{g/l}$)	
Trichloroacetic acid	0.08

triphenylphosphine, DFTPP) meets all the criteria specified in the method, with m/z ions ranging from 51 up to 443, with the most important relevant peaks at m/z values of 198 and 442. Other relevant ions are at m/z values of 51, 127 and 275. This MS method uses three different classes of MS analyzers, with magnetic sectors, quadrupole and ion traps. The LOD of Table 5.12 correspond to the use of an ion-trap MS.

In Table 5.13, from the different haloacetic acids determined by EPA methods, we have included trichloroacetic acid (TCA), which is very important also in the ED list of pesticides (Table 5.2). The method reported in Table 5.13 is also valid for other haloacetic acids and several chlorophenols. An NPS method, shown in Table 5.14 and still not considered an EPA method, was developed for ethylenethiourea (ETU). This is a relevant TP of maneb and related pesticides, is more toxic than the original pesticides, and has been found in water sources. In the next chapter of this book dealing with coupled column LC an elegant method is described for this compound, which can certainly compete with this NPS method.

TABLE 5.14

NPS METHOD 6 (SEE TABLE 5.4): DETERMINATION OF ETHYLENETHIOUREA (ETU) IN GROUND WATER BY GC WITH NPD

Summary of the method	The ionic strength and pH of a measured 50-ml aliquot of sample are adjusted by addition of ammonium chloride and potassium fluoride; the sample is poured into an Extralut column; ETU is eluted from the column with 400 ml of dichloromethane; the extract is solvent exchanged to ethyl acetate and concentrated to a volume of 5 ml
GC columns used	10 m \times 0.25 mm i.d. DB-WAX and confirmatory column 5 m \times 0.25 mm i.d. DB-1701
Minimum quantification limit ($\mu\text{g/l}$)	9.0

From the results reported from the NPS in collaboration with the EPA, several general comments can be made concerning the determination of pesticides in drinking water samples. (i) These methods describe in detail all the parameters for a good monitoring programme for pesticides and consist of the most complete studies so far published. They define, for example, the storage of samples, with preservation by adding HgCl_2 or monochloroacetate buffer, the use of replicate analyses after 0, 14 and 28 days of storage under refrigeration at 4°C ; the use of at least two different columns, one primary column and a secondary column of different polarity for confirmation purposes. When MS is used, only one GC column is employed, since the confirmation is achieved by MS, using ion-trap, quadrupole or high resolution analyzers. (ii) The NPS has established the monitoring of many TP's of pesticides and for the first time the names of the TP's are indicated in the survey. (iii) The development of micro-extraction LLE, SPE and GC-MS methods is being encouraged and is one of their strongest recommendations [27,28].

In the final report of the NPS, it was shown that DCPA acid metabolites were present in the highest percentage in community water wells and rural domestic wells, with an estimate of over 10 million people being exposed to this pesticide. However, very few are expected to be exposed to levels above the health advisory level. Other pesticides found in 0.1–6.4% of wells were: atrazine, simazine, prometon, lindane, ETU, bentazone and alachlor (hexachlorobenzene and dibromochloropropane, ethylene dibromide and dinoseb have had their registration cancelled by the EPA) [12].

5.4. SCA METHODS OF ANALYSIS

These methods are the Official Methods of the Department of Environment Drinking Water Inspectorate Standing Committee of Analysts (SCA). Many of them, also known as SCA methods, were discussed in two recent review articles [29,32]. Although there are also official methods of analysis of pesticides in water in several EC countries, these methods are not discussed here. Quite a difference still exists between such official methods of analysis, mainly based on LLE procedures, and other multi-residue methods based mainly on SPE techniques. The number of laboratories that use SPE techniques for the isolation and analysis of pesticides in water is certainly increasing in the Netherlands [15], Germany [16,33] and Italy [34,35]. Recent papers published in the different European countries show the lists of pesticides relevant to each country, and are slightly different from the Rhine basin to the Po river.

Only selected SCA methods for pesticides in water are compared with those used by the US EPA. One deficiency of the EC countries is that a body similar to the EPA does not exist in Europe, so official methods for all of Europe have not yet been developed. In this comparison, similarities and differences between the UK and the US methods are shown, with the hope that in the future we will be able to talk about common EC methods for pesticides in drinking waters.

When comparing the United Kingdom and the United States., two points need to be mentioned. First, with regard to the pesticides of interest (see Tables 5.1–5.4), there are many pesticides which are common. Secondly, as mentioned earlier, in Europe the levels for any pesticide have a limit of $0.1\ \mu\text{g/l}$ for drinking water requirements, which is a much lower value than most of the maximum concentration values fixed by the US EPA, which

are based on health advisory levels (see Table 5.5). So, in this sense the method development in Europe needs to achieve about one order of magnitude lower for detection than do the US EPA methods, thus causing more difficulties in monitoring a large number of pesticides. The efforts of different research groups working on the monitoring of pesticides in water are directed towards achieving the detection limits required by the EC (which should be at least $0.02 \mu\text{g/l}$) in order to quantify samples at $0.1 \mu\text{g/l}$. However, many of the UK methods of analysis still have LOD higher than the EC requirements, and only during the last few years has method development been carried out in different European laboratories to achieve such a goal [34,35]. Figure 5.5 shows the GC-MS separation of 50 pesticides from various chemical groups, i.e. OC, organophosphorus and organonitrogen. It corresponds to a screening method used in Italy for the isolation of priority pesticides from water samples at low LOD, below $0.1 \mu\text{g/l}$.

For the organophosphorus pesticides dichlorvos, dimethoate, malathion, parathion, fenitrothion, chlorfenvinphos, carbophenothion, pirimiphos-methyl and chlorpyrifos, an extraction method involving 25 ml of *n*-hexane and 50 ml of dichloromethane for 1 l of river or drinking water has been employed. The extracts are taken up in 1 ml of acetone, after evaporation of the dichloromethane. Afterwards, $1 \mu\text{l}$ is injected onto a GC with

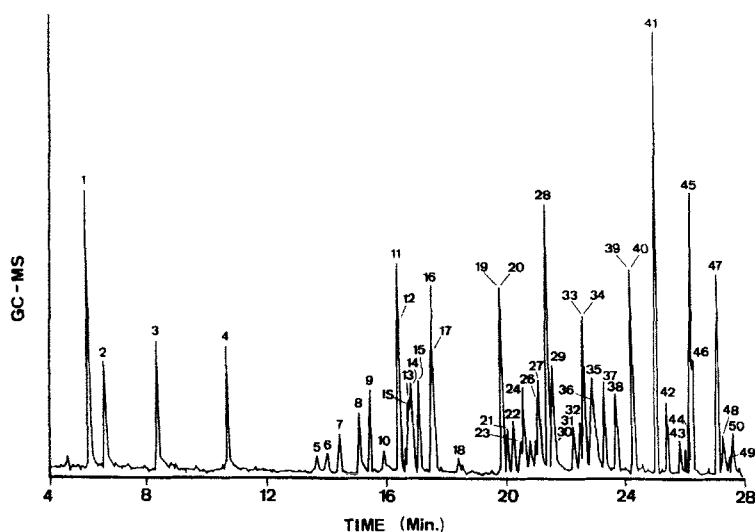


Fig. 5.5. GC-MS separation of 20 ng of: (1) dichlobenil, (2) EPTC, (3) butylate, (4) molinate, (5) cimoxanil, (6) de-isopropylatrazine, (7) de-ethylatrazine, (8) phorate, (9) trifluralin, (10) dimethoate, (11) lindane, (12) simazine, (13) atrazine, (14) prometon, (15) propazine, (16) trietazine, (17) terbutylazine, (18) diazinon, (19) heptachlor, (20) propanil, (21) parathion-methyl, (22) carbaryl, (23) simetryne, (24) alachlor, (25) ametryne, (26) metalaxyl, (27) prometryne, (28) aldrin, (29) linuron, (30) terbutryne, (31) metolachlor, (32) malathion, (33) chlorpyrifos, (34) parathion, (35) triadimefon, (36) tiocarbazil, (37) diphenamid, (38) captan, (39) methidathion, (40) α -endosulfan, (41) DDE, (42) β -endosulfan, (43) flamprop-isopropyl, (44) benalaxyl, (45) DDT, (46) pyrazon, (47) tetradifon, (48) azinphos-methyl, (49) fenamirol and (50) azinphos-ethyl. A capillary GC CP Sil 8 CB column was used. (Reproduced with permission from [34].)

flame thermionic or flame photometric detection (FPD) [36]. Although the first version of the method used packed GC columns, as in the US EPA methods, the SCA method now recommends the use of 25–50 m capillary columns of OV-1 or SE-54 [37]. However, the method is less specific and does not indicate the use of a confirmatory column. Like the US EPA, they also recommend that one should determine the organophosphorus pesticides as soon as possible, since they can degrade rapidly. The method is also based on LLE as with the EPA method (see Table 5.7) but uses two extracting solvents, *n*-hexane and dichloromethane, instead of dichloromethane alone in the EPA method. The use of a mixture with *n*-hexane allows better recovery of the more non-polar organophosphorus pesticides, such as chlorpyrifos, fenitrothion, carbophenothion and pirimiphos-methyl. It is worth mentioning that the SCA method does not find significant differences in the recovery values between water with high suspended solids and water with low suspended solids. The LOD varied between 0.04 and 0.8 $\mu\text{g/l}$. In order to compare these results with the US EPA method (Table 5.7), it should be mentioned that there are very few organophosphorus pesticides in the EPA method, since the degradation of the water solutions kept in the refrigerator occurred rapidly [14]. One of the compounds, dichlorvos, had a LOD of 0.04 $\mu\text{g/l}$ in the SCA method, which can be attributed to a different way of determining the LOD by baseline fluctuation and using a FPD, which is reported to usually be more sensitive to P [38].

The determination of triazine herbicides in drinking waters was based on an alkaline extraction (2 ml of ammonia) into dichloromethane (100 ml and 50 ml), then with concentration into 2 ml of methanol and injection of 5 μl into the GC-NPD. A 50 m WCOT column is recommended, containing Carbowax 20 M. A detection limit of 0.015 $\mu\text{g/l}$ is estimated for atrazine, simazine, prometryne, propazine and terbutryne [39]. The method does not differ considerably from the EPA report in Table 5.7 for the analysis of different organonitrogen and organophosphorus pesticides. One comment on this method is that it is not necessary to use such a long column as 50 m: with the EPA column the separation can be achieved by a 30 m column. A recently published modification of this method [40] was developed for the determination of the chlorotriazine TPs, such as deethylatrazine, deisopropylatrazine and hydroxyatrazine. With the use of a mixture of ethyl acetate/dichloromethane and 0.2 M ammonium formate, it was possible to increase the extraction recovery of the different chlorotriazine TPs.

The most extensive list of possibilities using the SCA methods has been published for the analysis of chlorinated acids [39]. The different methods, similarly to the US EPA (see Table 5.9) are based on the formation of derivatives, followed by GC-ECD determinations. Since the SCA method recommends a different derivatization for each of the chlorinated acid herbicides, it is interesting to show a summary of all the possibilities in Table 5.15. The methods indicated in this table generally use 1 l of water, an acidic extraction with diethyl ether, hydrolysis and derivatization with a final volume of 1 ml. A 5 μl sample is injected onto the GC which uses a fused silica wall-coated open tubular column, 25 m long, containing methylsilicone stationary phase. The methylation, indicated in Table 5.15 under method B, is more similar to the US EPA. In addition, the method for the acidic herbicides is also valid for other compounds, such as polychlorophenols. As an example, the formation of pentafluorobenzyl esters is shown in Fig. 5.6, for a standard herbicide mixture (A) and of river-water extract (B). The LOD was in the range of 0.05 $\mu\text{g/l}$.

An important remark is that DCPA (Dacthal or Chlorthal) and DCPA acid metabolites are not included in the SCA methods of analysis, probably due to the fact that the parent compound is practically not used in Europe, and no evidence was found of its use or any levels detected in monitoring of ground-waters within different European countries [5]. In contrast, such compounds are the most relevant herbicides detected in the NPS, as we have seen previously. DCPA acid metabolites can be analyzed by Method B of Table 5.15, which is quite similar to the EPA (Table 5.9). This compound and its TPs are rather stable in soil, with half-lives of 100 and 365 days, respectively [12]. The monitoring of this acidic herbicide is noticeably different between the United States and Europe, since it can affect the water supply of more than 10 million people within the United States, whereas no incidence has apparently been observed in Europe.

TABLE 5.15

SCA METHODS FOR THE DETERMINATION OF CHLORINATED PHENOXY ACIDS IN WATER

Method A		Extraction, hydrolysis, butylation and GC-ECD			
Preferred for		2,4-D, 2,4,5-T and Dalapon			
Limit of detection ($\mu\text{g/l}$)		For 2,4-D, 0.024 and for 2,4,5-T, 0.004			
Method B		Extraction, hydrolysis, methylation and GC-ECD			
Preferred for		2,3,6-Trichlorobenzoic acid (TBA), Dicamba, polychlorophenols			
Also suitable for		2,4-D, 2,4,5-T and dichlorophenols			
Limit of detection ($\mu\text{g/l}$)					
2,4,6-Trichlorophenol	0.07	2,4,5-Trichlorophenol	0.2		
2,3,4,6-Tetrachlorophenol	0.02	Pentachlorophenol	0.02		
2,3,6-TBA	0.0005				
Method C		Extraction, perfluorobenzylation, GC-ECD			
Preferred for		MCPA, MCPB and MCPP (Mecoprop)			
Also suitable for		Dicamba, TBA			
Limit of detection ($\mu\text{g/l}$)					
MCPB	0.11	Dicamba	0.10	MCPA	0.08
2,3,6-TBA	0.08	2,4-D	0.14	2,4,5-T	0.11
MCPB	0.10				
Method D		Extraction, hydrolysis, methylation, GC-MS			
Preferred for		MCPA, MCPB, MCPP, 2,4-D and 2,4,5-T			
Limit of detection ($\mu\text{g/l}$)		1 (two suitable ions for each analyte are used in the multiple ion detection)			
Method E		Extraction, hydrolysis, nitration, methylation and GC-ECD			
Preferred for		MCPA, MCPB and MCPP			
Limit of detection ($\mu\text{g/l}$)		0.004 for MCPA			

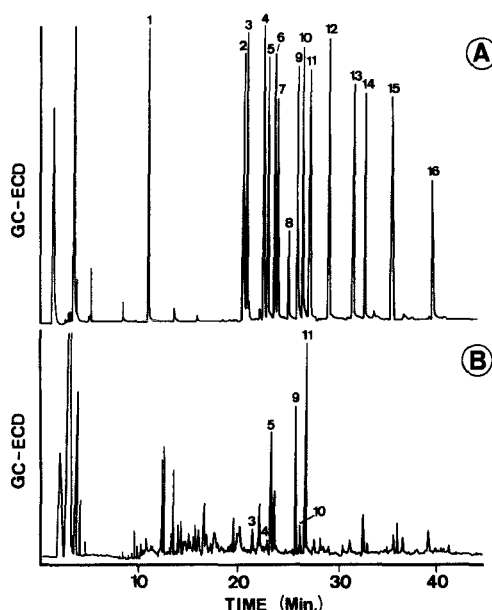


Fig. 5.6. GC-ECD chromatograms of: (A) 1-ng injection of standard herbicide of pentafluorobenzyl (PFB) derivatives of (1) pentafluorophenoxyacetic acid, (2) 4-CPA, (3) MCPP, (4) dicamba, (5) MCPA, (6) 2,4-DP, (7) 2,3,6-TBA, (8) TBA, (9) 2,4-D, (10) bromoxynil, (11) 2,3-D, (12) silvex, (13) 2,4,5-T, (14) MCPB, (15) 2,4-DB, (16) picloram. (B) 1 l of river-water extract obtained after LLE with the residue concentrated down to 0.5 ml with an injection of 2 μ l. A capillary GC DB-5 column was used.

For the analysis of dithiocarbamates and related compounds such as maneb, mancozeb, nabam, zineb, ferbam and thiram, the water sample is heated with acid in the presence of stannous chloride and 2,2,4-trimethylpentane (iso-octane). The carbon disulphide formed dissolves in the iso-octane and is determined by GC-FPD [41]. The LOD is 0.48 μ g/l (0.84 μ g/l as maneb). A great disadvantage of this method is that the result corresponds to the total of the compounds listed, together with any others that undergo the same reaction. During the last few years an elegant LC method was developed, based on post-column complexation of the dithiocarbamates with finely divided copper, to form a coloured complex [42].

Synthetic pyrethroids such as permethrin, cypermethrin, alphacypermethrin, fenvalerate and deltamethrin are determined using solvent extraction of 1 l of water with 100 ml of hexane, with clean-up methods involving Florisil (or aminopropylsilica, or alumina) and with further analysis by GC-ECD and confirmation by GC-MS with negative chemical ionization (NCI) [43]. The method allows a LOD of 0.005 μ g/l for each of the studied compounds, which complies with EC directives. The columns used are of DB-5 or SE-54, 30 m long \times 0.33 mm id. This method is the first that recommends the use of NCI in the selected ion monitoring mode: this is a difference from the EPA methods, which only use GC-MS in the conventional EI operation. In this case also, the method indicates that when MS facilities are not available, another capillary column coated with a different stationary

phase should be used for confirmation purposes. This method is clearly more specific and advanced than the EPA method, since: (i) more pyrethroids are analyzed; (ii) there are three options for the clean-up steps; (iii) the use of NCI is recommended, which is not yet in the EPA methods. A summary of the method is shown in Table 5.16.

To summarize the general similarities and differences between the US EPA and SCA methods, it can be mentioned that the SCA methods: (i) place less emphasis on the use of confirmatory columns (which avoid false positives), surrogates and internal standards; (ii) the compounds to be monitored are far fewer than the 126 in the NPS-EPA list, so fewer screening methods are available; (iii) do not monitor DCPA, the most important herbicide in the United States, and its TP. Although there are similarities in the compounds and methods between both, the US EPA and SCA; (iv) offer practically no information for the analysis of TPs, whereas the NPS has already introduced a method that follows up to 25 TPs; (v) two of the methods (for pyrethroids and phenoxy acids) are superior to the EPA, since confirmation by GC-MS with NCI is shown for an extended list of pyrethroids, and for phenoxy acids they offer three different alternatives for derivatization, depending on the compound to be analyzed, together with GC-MS confirmation; (vi) for triazines and organophosphorus pesticides, there are few differences compared to the EPA.

To conclude this comparison, we can critically state that all the official methods of analysis can be improved. Certainly, the determination of dithiocarbamates is not elegant with the SCA method. As already indicated in the latest EC report on pesticides in drinking water [5], analytical methods with low levels of detection need to be developed for this group of pesticides, such as maneb, mancozeb, ziram and others for which no suitable analytical method appears to exist. In general, it can be stated that, within Europe, method development for the determination of pesticides in drinking water is much more needed and urgent than in the United States, because of the more restrictive measures for the quality of drinking water. Another general remark, which applies to all the methods for

TABLE 5.16

SCA METHOD FOR THE DETERMINATION OF SYNTHETIC PYRETHROID
INSECTICIDES IN WATERS BY GAS LIQUID CHROMATOGRAPHY

Summary of the method	1 l of water sample is extracted with 100 ml of <i>n</i> -hexane by shaking during 1 min; once the extract is concentrated, Florisil or aminopropylsilica cartridges are used for clean-up; analytical determinations are carried out by GC-ECD, with further confirmation by GC-MS with negative chemical ionization (NCI)		
GC column used	60 m × 0.25 mm i.d. WCOT capillary column with DB-5 (0.25 μm thickness) stationary phase		
Limits of detection (μg/l)	0.01		
Suitable ions (<i>m/z</i>) for GC-MS NCI confirmation			
Cyhalothrin	205, 241	Permethrin	207, 209
Cyfluthrin	207, 209	Cypermethrin	207, 209
Deltamethrin	79, 81		

water, is that too many official methods are still based on LLE, with its problems of solvent disposal. The future within Europe will certainly see the development of screening methods for a wide range of pesticides, based on SPE principles [34,35], with LOD down to or better than $0.02 \mu\text{g/l}$, thus permitting the quantitation of $0.1 \mu\text{g/l}$ of each individual pesticide.

5.5. OTHER GC METHODS

Capillary gas chromatography (GC), in combination with selective detectors, mainly nitrogen-phosphorus (NPD), electron capture (ECD), flame photometric (FPD) or mass spectrometry (MS), is still the most common technique for the determination of environmental pesticide residues in water. This is clear from the official methods of analysis discussed above. The low LOD, high selectivity and affordability of GC instrumentation is rather appealing to most laboratories involved in pesticide residue analysis. Several reviews on the use of GC-NPD, GC-ECD and GC-MS have been published [44,45]. Recently, a book with various GC and LC approaches for multi-residue analysis and for specific groups such as carbamates and organophosphorus and organonitrogen compounds has been published [46].

Examples of the use of GC-NPD for the routine determination of organophosphorus and organonitrogen pesticides in water samples, following a multiscreening method similar to EPA Method 507 (Table 5.7) have been reported elsewhere [7,47,48]. Examples are given of method development for the determination of organonitrogen and organophosphorus pesticides in water: ametryne, atrazine, atraton, prometryne, metolachlor, fenitrothion, fenthion and parathion-methyl were determined. However, in recent years, methods based on SPE, instead of the conventional dichloromethane LLE, have been developed, as already mentioned earlier. Examples of the use of SPE, mainly using C-18 silica cartridges followed by GC-ECD, are available for atrazine, alachlor, metribuzin and metalachlor [49]. Detection by GC-NPD has been reported for carbaryl, carbofuran, fonofos, parathion, alachlor, cyanazine and metribuzin [50,51] and by GC-alkali flame ionization for several organophosphorus compounds, pyridafenthion and tetrachlorvinphos, and triazines such as atrazine and prometryne [52].

The use of SPE methods for the isolation of pesticides from water has been of increasing interest in recent years and will probably replace conventional LLE, not only in research laboratories where it is already quite common, but also in government laboratories where conventional LLE procedures are still much in use. The application of SPE approaches has been expanded recently by the use of novel products, which are Empore extraction disks containing either C-18 or polystyrene-divinylbenzene material. These can be used in a similar way to the cartridges but with the major advantages of shorter extraction times, owing to the lack of channeling, and faster mass-transfer owing to smaller pore sizes ($8 \mu\text{m}$ versus $40\text{--}60 \mu\text{m}$). They have been applied to the determination of various pesticides in water matrices, with analysis by GC-ECD [53]. Recently, the Empore disks have been coupled on-line with GC-NPD for the direct analysis of 2.5 ml of water samples containing various organophosphorus pesticides and have achieved LOD of $0.1 \mu\text{g/l}$ [54].

Electron capture detectors, the most commonly used to analyze classical chlorinated pesticides such as DDT and endrin, which are not discussed here, are resorted to for molecules that contain chlorinated groups, for example atrazine, chlorpyrifos, metoxychlor and trifluralin. GC-ECD is the method of choice for the identification of several unstable pesticides which need derivatization prior to determination. Examples, with the derivatives, are carbamates (trichloroacetyl), chlorinated phenoxy acids (pentafluorobenzyl or diazomethane esterification) or urea pesticides (heptafluorobutyric) [22,24,55–57]. Some of these methods include subsequent confirmation of the derivatives by GC-MS [22,24,57]. Most of the derivatization methods developed are for the acidic herbicides and usually refer to previous EPA methods (see Table 5.9). Such methods are usually more rapid [55] or introduce refinements in sample clean-up, confirmation of compound identity and quality assurance [57]. For instance, for the detection of acidic herbicides at the 0.02–0.05 $\mu\text{g/l}$ level, the pentafluorobenzyl derivatives are recommended rather than the classical diazomethane reagent [57], which lacks the sensitivity required by the EC for monitoring pesticides in drinking water. An example of the use of pentafluorobenzyl derivatives is indicated in Fig. 5.6.

In order to avoid “false positives” in the determination of pesticides in water samples, confirmatory techniques are needed. As we have seen in the previous EPA methods, such confirmation is usually achieved by injecting the sample extract onto a second column of different polarity. However, such comparisons do not constitute a foolproof means of confirmation, although they do provide strong evidence for identification of unknowns. Another way of carrying out such confirmation using a second column is to apply the so-called two-dimensional capillary gas chromatography. In this way, two columns of different selectivity are combined in such a way that the eluate fraction can be directly transferred from one column to another. The different aspects, involving valve-switching versus pneumatic switching, pneumatic effluent transfer and the different modes of operation (cut, straight and backflush) have been discussed in a review [58]. Examples of the use of linked response data from parallel FPD and ECD detectors with retention data from linear temperature programming [59], and even with the use of three selective detectors (FPD, NPD and ECD), have recently been published [60]. A second approach for confirmation of pesticide residues in environmental matrices is the use of chemical derivatization, a technique that has found substantial use when other means of confirmation were not available. Examples of the reagents and chemical reactions used for organophosphorus pesticides have been reported recently [38]. The formation of a derivative, for example by trifluoroacetylation, means that the original pesticide disappears and the new derivative, with a shorter retention time, can be confirmed.

GC-MS is the most commonly used confirmation technique and in many cases is also applied for analytical determinations. The increasing importance of this approach to the analysis and confirmation of pesticides in water is linked to the fact that the EPA and SCA methods previously discussed have already implemented GC-MS in some of their protocols, with a tendency to include MS confirmation in future or modified new analytical methods. The use of the EPA Method 525 (see Table 5.12), based on SPE with either cartridges or disks, has also been evaluated in the literature [31], and showed low interferences from the disks in the MS background spectra, with a considerable increase in the speed of routine analysis over the cartridges, as mentioned earlier. A screening method based on the use of SPE, with a variety of materials (C-18, NH_2 and Ph) and GC-MS de-

termination was developed by the Mario Negri Institute [34] and applied to 50 pesticides at the sub-microgram per litre level, including atrazine, propanil, trifluralin, chlorpyrifos and tetradifon. An example was given in Fig. 5.5. The isolation of several triazines was evaluated using SPE cartridges of Sep-Pak C-18 [61] and by using a styrene-divinylbenzene copolymer such as PLRP-S [62] in combination with GC-MS using various ionization modes such as EI, PCI and NCI. The use of XAD-2 and XAD-7, in combination with GC-MS with an ion trap analyzer, has been reported for several pesticides, including alachlor, diazinon and metribuzin [63]. The use of C-18 cartridges in combination with isotope dilution GC-MS has been reported for the maize herbicides, with LOD of $0.05 \mu\text{g/l}$ [64].

Applications of the use of LLE approaches, based on dichloromethane extraction similar to the EPA method for organonitrogen (Table 5.7), in combination with quadrupole GC-MS [7] and ion-trap GC-MS [65] have been reported for common maize herbicides, such as atrazine, alachlor, metolachlor and simazine.

Although most of the confirmation is carried out by using GC-MS with EI, NCI is increasingly recommended, as was already observed in the SCA method for pyrethroids. A recent work also demonstrated the use of this selective technique for the determination and confirmation of acidic herbicides such as MCPA and dicamba, in natural waters at levels of $0.02 \mu\text{g/l}$ [66]. So far in this chapter, the methods for determination of organometallic compounds used as pesticides have not been mentioned. In a recent review article, references about methods for the determination of these compounds are given [44]. It is worth indicating that a common method for the determination of organometallic compounds, for example butyltin, uses a dichloromethane and tropolone extraction. Levels of 5 ng/l can be achieved for tributyltin in sea water using GC-MS with an ion-trap detector [67]. Figure 5.7 shows examples of the GC-MS analysis of

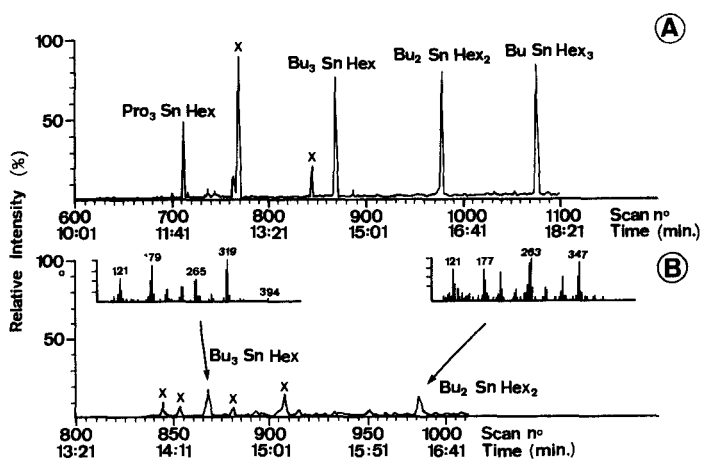


Fig. 5.7. GC-MS chromatogram of: (A) standard sample containing propyl- and butyltin chlorides hexylated and cleaned-up on silica gel column; (B) butyltin compounds extracted from natural sea-water spiked with $2 \mu\text{l}$ of Bu_3SnCl and Bu_2SnCl_2 . Marked peaks (x) are impurities containing no tin. (Reproduced with permission from [67].)

(A) a standard sample and (B) an extract of butyltin compound from natural sea-water samples.

5.6. CONCLUSIONS

From the methods of analysis reported in this work, it is clear that there is still a divergence between the official methods of analysis used in the different countries and the newest techniques used in research and private laboratories. The progress in incorporating modern analytical methods into official methods is slow, as can be seen in the use of SPE techniques, capillary GC columns or MS confirmation. However, there have been some advances, especially within the US EPA as compared with the European official methods of analysis.

Future recommendations for work to be carried out using GC methods will be as follows. (i) There is a need to develop off-line SPE techniques based on new adsorbent types, e.g. styrene-divinylbenzene copolymers, carbon types, or Empore disks, in combination with GC-MS using selective ionization methods such as NCI. In a similar way to the use of NCI for pyrethroids in the SCA methods of analysis, it could be a technique recommended for confirmation of organophosphorus pesticides exhibiting an electron-withdrawing structure, such as a parathion-group. (ii) The development of specific methods of analysis for particular pesticides will be of interest, like the work of the US EPA for unusual pesticides (e.g., endothall). In this sense, the EC has defined the need for developing analytical methods at a low level of detection for difficult pesticides such as maneb, ziram and metham-sodium, among others. (iii) The development of immunochemical methods, radio-immunoassay and enzyme-linked immunoassay will be of interest in the future, specially when linked to chromatographic techniques. So far, most of these techniques have been used for the detection of pesticides in waters, and in some cases have been compared with conventional chromatographic methods and show good correlation for quantitation purposes. (iv) There is a need for validation studies when modern techniques are incorporated into the official and/or routine methods of analysis. This will be the case for the use of GC-MS for quantitation purposes.

From the above conclusions, it is clear that much work still needs to be done on the analysis of pesticides in water samples. This particular field of research is also changing each year, since new pesticides are being developed to substitute those which are more toxic or have led to widespread contamination. This is the case, for example, with atrazine, which is being slowly replaced in some countries by terbutylazine and/or propazine. In this sense, analytical developments need to be continuously carried out to determine the new pesticides and the increasing amounts of toxic TP's that are being released into the different types of environmental waters.

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Chapter 6

Coupled-column reversed phase liquid chromatography as a versatile technique for the determination of polar pesticides

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6.1. INTRODUCTION TO PESTICIDE RESIDUE ANALYSIS

The basic purpose of pesticide residue analysis (PRA) is to provide reliable and cost effective methods for the identification and quantification of more than 400 trace analytes occurring in a variety of matrices.

Before 1960, most analyses were conducted for individual pesticides using relatively unspecific methods including spectrophotometry, total halogen methods (for chlorinated pesticides) and biochemical techniques involving the inhibition on thin layer plates of the enzyme cholinesterase (for organophosphorus insecticides) or the fungus *Penicillium*

cyclopium (fungicides). Thus, it is interesting to notice the present introduction of biological techniques based on immunoassays in PRA.

The field of PRA was revolutionized in the late 1960s by the introduction of gas chromatography (GC). Application of this technique made it possible to separate a number (approx. 15 on packed columns) of pesticides in one run, initiating the development of multi-residue methods (MRMs). The introduction of capillary GC in the late 1970s and the availability of sensitive and selective GC detectors contributed largely to its wide application in PRA. The favourable features and the fact that regular PRA laboratories are, for historical reasons, better equipped with GC, make it still the major analytical technique in PRA.

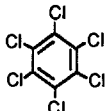
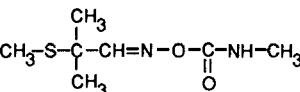
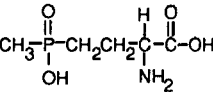
Since the introduction of liquid chromatography (LC) into the field of PRA in the late 1970s, its application is growing fast. It is especially valuable for the determination of pesticides that cannot be analysed directly by GC. This may result from their poor volatility, polarity and/or thermal instability, as is the case for chlorophenoxy herbicides, carbamate insecticides and phenylurea herbicides. These analytes can be separated and detected efficiently by LC while GC needs a derivatization step prior to the chromatographic analysis.

From an economic point of view, the availability of MRMs which can quantify more pesticides in one analytical procedure is important. The development of MRMs usually involves three major considerations.

- (i) Because it deals with liquid and solid substances of wide variety and different compositions, PRA is faced with the investigation of a large diversity of samples. Current MRM methodology is mainly focussed on the development of dedicated pesticide/matrix methods, and tends to neglect the multi-matrix aspect. In order to obtain methods with a broader scope, more efforts should be made towards the development of multi-matrix techniques.
- (ii) The levels of determination are substrate dependent. Maximum residue levels (MRLs) of pesticides are regulated by the Dutch Administration [1] in agreement with the directives of the European Committee (EC) and complying with Codex recommendations. Recently, Wessel [2] reviewed the Codex- system for the establishment of MRLs. The levels are based on scientific evaluations which estimate an acceptable daily intake for a pesticide, and the expected level of residue remaining in food when it is used according to good agricultural practice. This means that, even for one pesticide, MRL ranges can cover several orders of magnitude, which is a severe drawback for a generally applicable MRM methodology. In environmental samples and drinking water, the MRMs are generally much lower. The EC directive for drinking water [3] states that the concentration of pesticides and related products should not exceed the level of $0.1 \mu\text{g/l}$ (ppb) for individual compounds and $0.5 \mu\text{g/l}$ for total pesticides. This requires methods of analyses about 1000 times more sensitive than for foodstuffs.
- (iii) The large variety of physico-chemical properties of pesticides, e.g. in their molecular weights, stability and polarity, forms a large problem for MRM development. The large differences in polarity make a simultaneous chromatographic analysis of different pesticides difficult or even impossible. Table 6.1 gives examples of the large differences between the polarities of pesticides.

TABLE 6.1

DIFFERENCE IN POLARITY OF SOME WELL-KNOWN PESTICIDES

Pesticide	Formula	Solubility in water (mg/ml)	P _{OW}
HCB		<0.1	4×10^5
Aldicarb		6×10^3	12
Glufosinate		1.4×10^6	<0.1

^aOctanol/water partition coefficient.

Analytical methodology has to face new pesticides as they are brought onto the market and the changing patterns of use of existing ones. Until the 1980s, the major analytical aim was to investigate the possible occurrence of pesticide residues and their metabolites in food which has, at any stage of its growth or production, been treated with pesticides. Analytical efforts were primarily directed to the detection and determination of pesticides in food, and this will be necessary as long as pesticides are being used and new ones are being marketed. For example, one can observe a shift from "long-life" pesticides such as organochlorine compounds, to the use of "short-life" pesticides such as *N*-methylcarbamate insecticides. Additionally, over the years concern has been extended to the environment and this requires additional sensitive and selective analytical methods for materials such as herbicides and soil fumigants which are not directly applied to foodstuffs. These developments will continue and so will the development and improvement of the quality, scope and cost-effectiveness of analytical methods. Hence, the changing field of PRA requires not only dedicated MRMs but also the availability of flexible strategies for method development.

An outline of methods used today in The Netherlands for the determination of pesticides in foodstuffs [4] is schematically presented in Fig. 6.1. Using available MRMs, about half of the total number of registered pesticides can be determined in foodstuffs. This indicates that the assay of about 200 pesticides is still done with single residue methods (SRMs). A similar situation is encountered for the analysis of pesticides in environmental samples. As indicated in Fig. 6.1, the remaining group mainly deals with moderately polar to very polar pesticides, which can generally be separated more efficiently by using LC than GC. Reversed phase liquid chromatography (RPLC) is particularly convenient for the chromatographic analysis of polar compounds. Hence, from a chromatographic point of view, it is appropriate to study the feasibility of RPLC for the devel-

opment of MRMs for polar pesticides. An additional advantage of RPLC compared to GC is its absence of laborious derivatization steps, which makes it more attractive for the development of SRMs for polar pesticides.

A general scheme of the analytical procedure in PRA is depicted in Fig. 6.2. For solid samples, PRA usually involves the application of five steps: (1) isolation (extraction) of the pesticides from the sample by extraction with an organic solvent; (2) concentration/evaporation of the organic solvent; (3) clean-up of the concentrate prior to instrumental analysis; (4) evaporation of the collected fraction and its dissolution in a small volume of solvent which is compatible with the chromatographic system; (5) instrumental analysis of an aliquot of the final extract and data handling. For aqueous samples, the second step can be avoided by the application of solid phase extraction (SPE) systems. However, for the processing of solid samples, steps 1–4 are mostly carried out manually [4]. These manual steps are tedious, laborious and subject to errors such as losses of analytes by evaporation or adsorption during sample handling. Improvement of the sample throughput and the overall performance of the method can be accomplished by automating the steps shown in Fig. 6.2. Without the application of expensive robotic instruments, automation of step 1 for solid samples is impossible. Therefore, the advantages of automation in PRA for foodstuffs, soil and other solid samples will largely be focused on step 3, the clean-up procedure (see Fig. 6.2). This involves a (pre)separation of the analytes from the large amount of matrix interferences, and is usually performed using low resolution chromatography such as open column liquid chromatography, SPE on disposable tubes, or gel permeation chromatography (GPC).

Steps 3–5 can be combined and automated in a cost-effective way in commercially available instrumentation which uses coupled chromatographic systems. Such systems can also provide a considerable enhancement of both selectivity and sensitivity [5].

The potential and applications of coupled chromatographic systems for the analysis of pesticides is discussed in the next session.

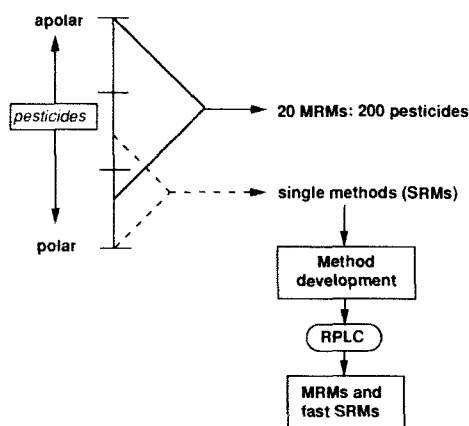


Fig. 6.1. Outline of existing pesticide residue analysis methodology [4]. For further explanation, see text.

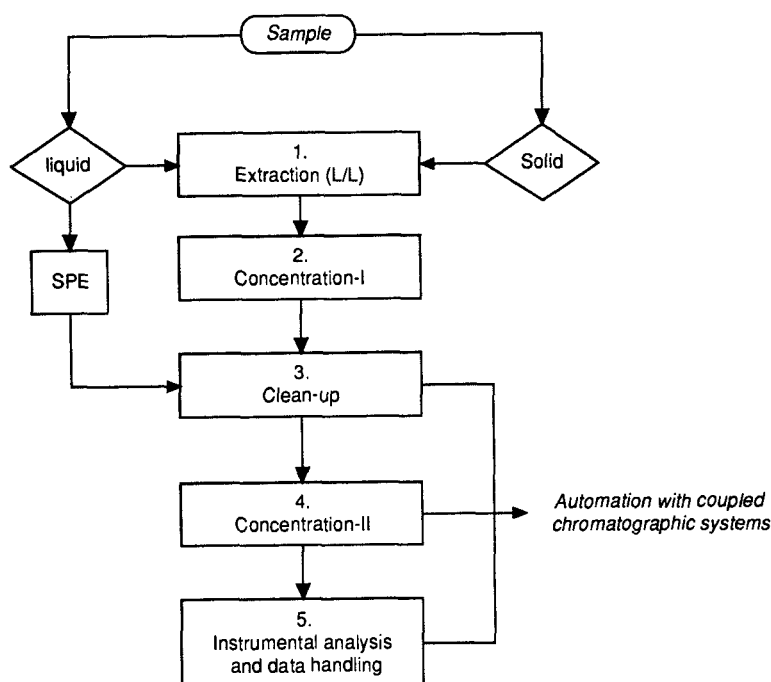


Fig. 6.2. Scheme of the procedure generally applied in PRA.

6.2. COUPLED CHROMATOGRAPHIC TECHNIQUES

6.2.1. Multi-dimensional chromatography

Multi-dimensional (MD) chromatography was originally developed for planar two-dimensional (2D) chromatography. Chromatography with two independent separation displacements in two different directions is a powerful technique for greatly enhancing the peak capacity and resolution of the analytes in complex mixtures [6]. The multiplicative law for the two-dimensional peak capacity with the enormous increase in resolving power gained in going from one-dimensional to two-dimensional TLC systems has been demonstrated [6–8].

In PRA, basically using LC and/or GC for the separation of complex mixtures, MD separation usually implies the application of two or more columns in which a fraction from one column is transferred to another by means of an interface. In order to approach the separation power of planar MD systems, the two chromatographic systems should have different selectivities which will result in two orthogonal separations.

However, in true orthogonal systems, the resolution gained in one column can be partially or entirely nullified by a different migration order in a subsequent column. This nullifying effect is demonstrated by noting [5] that the difference δt_r in the retention time of two analytes for the tandem system is the sum of the two increments in δt_r for the individual columns:

$$\delta t_r = \delta t_{r1} + \delta t_{r2} \quad (6.1)$$

The contributing increments δt_{r1} and δt_{r2} may be positive or negative. Therefore, they may either reinforce or nullify the separation. In fact, one must always be aware that two-dimensional coupled column systems may show a separation power in the first column which can operate in the same or opposite direction of the second. This effect is illustrated in Fig. 6.3, showing the RPLC and NPLC separation of synthetic pyrethroids. The imaginary coupling of this orthogonal separation mechanism will undoubtedly lead to a large reduction in the overall separation in comparison to a one-dimensional (column) separation.

In order to prevent a nullifying-effect, the application of coupled-column systems with orthogonal separation mechanisms requires preferably the use of small volumes for transferring the analyte fraction from the first column to the second. In fact, using a MD system with highly efficient separation columns renders MRMs unlikely because of the inherent separation power of the first column.

The potential gain in selectivity of coupled-column systems which use separation mechanisms with similar selectivities, e.g. RPLC-RPLC or GC-GC, will be less than for orthogonal systems. However, interfacing of such coupled-column systems is usually easy and, as far as the resolution between analytes is concerned, the separation power of the first column can largely be applied without limiting the transfer volume.

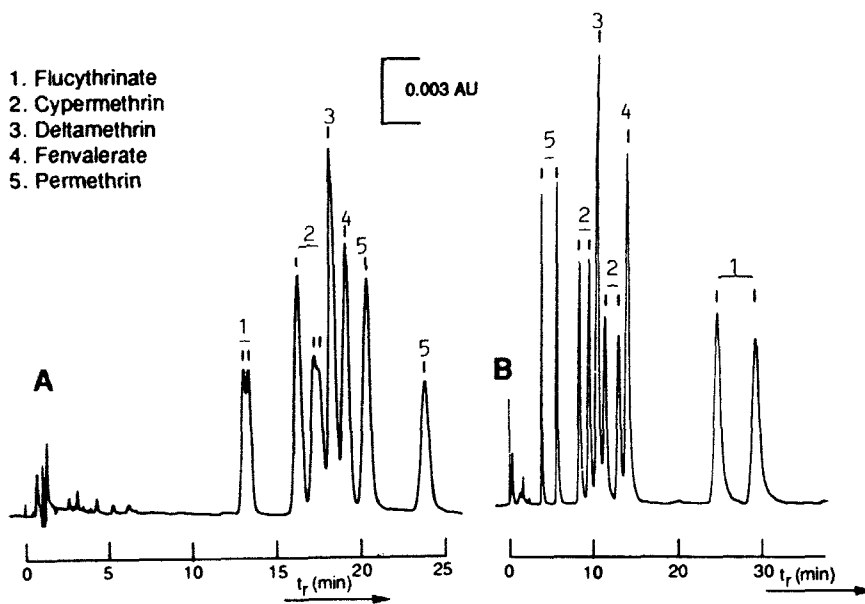


Fig. 6.3. Orthogonal HPLC separations of synthetic pyrethroids. (A) RPLC separation on a 150×4.6 mm i.d. column packed with $5 \mu\text{m}$ Hypersil ODS C-18 and a mobile phase of acetonitrile/water (70:30, v/v) at 0.8 ml/min. (B) NPLC separation on a 150×4.6 mm i.d. column packed with $5 \mu\text{m}$ of Lichrosorb Si-60 and a mobile phase of hexane/di-isopropyl ether (97.5:2.5, v/v) at 1.5 ml/min. Injected amount: 40–80 ng per analyte. UV detection at 229 nm.

Applications of various coupled chromatographic techniques encountered in PRA are discussed in the next sections.

6.2.2. GC-GC

GC column switching was applied in the early 1970s [9,10] using the approach originally described by Deans [11]. Today there are hardly any practical applications in pesticide residue laboratories. This can partly be attributed to the fact that the technique came onto the market just before the widespread introduction of capillary GC using fused silica capillary columns. Most of the research in the development and improvement of residue analytical methodology has been aimed at the implementation of capillary GC.

Important applications of GC-GC can be found in the analysis of organic non-polar micro-pollutants such as PCBs [12,13] and dioxins [13], which pose a serious separation problem because of their similar properties. These problems exist to a lesser extent in the analysis of pesticide residues. However, new trends in the pesticide market towards the production of optically active enantiomers instead of racemic mixtures, might revive the interest in GC-GC. The coupling of non-chiral columns to chiral columns seems to be a very suitable solution to solve the separation problems introduced by this trend [14,15].

6.2.3. LC-GC

Coupled LC-GC is becoming a well established technique for the determination of contaminants in food. The introduction of an interface that can transfer larger amounts of apolar organic solvents from LC to capillary GC initiated the development of a number of elegant applications of LC-GC in food, environmental and hydrocarbon analysis [16]. Today, two transfer techniques are commonly used: concurrent solvent evaporation with a so-called loop type interface and the retention gap techniques, including partially concurrent solvent evaporation, which employ an on-column interface [16]. Of these techniques, concurrent solvent evaporation using a vapour exit is the most simple and powerful for the injection of large volumes. With this technique, illustrated schematically in Fig. 6.4, volumes of up to 10 ml of organic solvent can be injected into a capillary GC, and acceptable chromatographic peak shapes are still obtained [17]. It is interesting to note that one of the first applications of on-line (NP)LC-GC was published in 1980 and dealt with the determination of the herbicide atrazine in sorghum at the 0.2 ppm level [18]. At that time only a small part (2 μ l) could be transferred by a GC autosampler for conventional vaporizing injection. Recently, atrazine in water could be assayed at the 5 ppt level by sampling 10 ml of the water onto the C-18 column of the (RP) LC-GC system and using a loop type interface to transfer the atrazine fraction (150 μ l) to the capillary GC column [19]. Other examples of applications of coupled LC-GC in pesticide residue analysis are the determination of folpet in hops [20], the assay of CGA 80000 in biological material [21], the analysis of dicamba (acidic herbicide) in tobacco leaves using a derivatization step followed by LC-GC [22] and determination of the conventional organochlorine pesticides and PCBs in various type of samples [23,24].

These applications demonstrate clearly the power of LC-GC in the high selectivity of the multi-dimensional technique. However, this important feature is not so favourable for

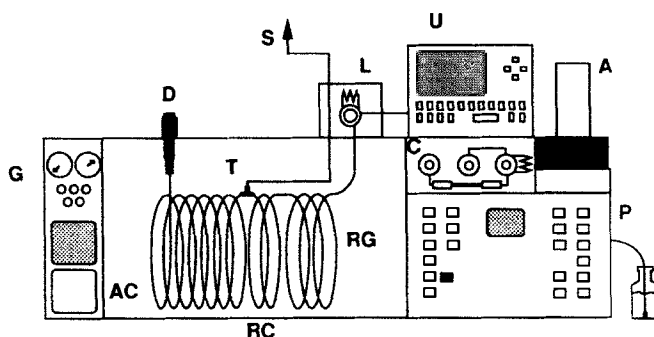


Fig. 6.4. Schematic representation of an LC-GC system. A, autosampler; P, LC-pump; C, LC column-switching valves; U, LC-UV detector; G, gas chromatograph; L, loop-type interface; RG, retention gap; RC, retaining precolumn; AC, capillary separation column; S, solvent vapour exit; D, GC-detector.

MRMs since group separations of pesticides by any LC technique are highly unlikely because of their widely different physico-chemical properties. Therefore, first-separation systems with a low separating power are more promising for GC-coupling than systems with high separating power.

This approach has successfully been elaborated in LC-GC using miniaturized precolumns [25,26], on-line membrane disk extraction [27] or disposable SPE cartridges included in a completely automated sampling system coupled on-line to the GC [28]. Because of the high separation power of capillary GC and the availability of sensitive and selective detectors, the application of flow injection analysis (FIA) seems promising. This is particularly so for the analysis of aqueous samples where sometimes only an isolation step is needed before the introduction of the sample in a GC, rather than a clean-up technique. Reports are available on the coupling of GC with an on-line extraction system for the automated sample pretreatment of aqueous samples and then analysis of chlorinated pesticides [29], aromatic hydrocarbons [30], halocarbons [31], nitrogen-containing pesticides [32] and organophosphorus pesticides [33]. A similar approach has been used for interfacing reversed phase LC to capillary GC [34].

GPC-GC seems a very logical candidate for application in PRA. It offers the possibility of separating the analytes in one, relatively small, fraction from macromolecular interferences of the sample. Unfortunately, GPC methodology is still faced with the problem of tailing (partial co-elution) of the fat peak from the GPC column [35], which hinders the determination of pesticides at low levels in fatty matrices.

The LC-GC applications discussed above all deal with the determination of rather non-polar pesticides, i.e. analytes that elute properly on a GC column. A promising technique for the analysis of polar analytes uses the on-line derivatization of analytes prior to the introduction to GC [36]. Recently, this technique has been reported [37] for the on-line determination of acidic pesticides in water using a two-phase catalysed reaction for the derivatization of the analytes with pentafluorobenzyl bromide.

6.2.4. LC-LC

The first application of column-switching LC [38] was reported in 1973. It was demonstrated that with the application of a one-pump system, a simple three-port switching valve and a low and a high capacity column, the total time of chromatographic analysis could be reduced considerably. It is noteworthy that the pioneers in this field estimated that such a set-up should be applicable to trace analysis.

Today, two different modes of column switching are usually encountered: PC-LC and LC-LC. The PC-LC (precolumn switching) method deals with the coupling of a low and a high resolution column, while LC-LC (coupled-column LC) makes use of two high efficiency separation columns. By applying different modes of separation, namely multi-dimensional (MD) chromatography, one can also discriminate between MD PC-LC and MD LC-LC.

The introduction of reversed phase precolumn switching (PC-LC) for the direct sampling of aqueous samples [39–41] resulted in a real breakthrough in column-switching applications. Particularly in the field of biomedical analysis, PC-LC appeared to be very useful for the on-line (automated) analysis of drugs and metabolites in liquid biological samples [42–44]. As well as good sample compatibility with aqueous phases used in RPLC, the successful use of PC-LC in biomedical applications is well suited to the concentration of the target analytes in body fluids at the sub-ppm level ($\mu\text{g/ml}$). At this level, sufficient sensitivity is usually obtained for the direct injection of 0.1–0.5 ml of sample, which is compatible with fully automated procedures for the processing of large sample series with commercially available autosamplers. However, trace analysis of pollutants at the sub-ppb level (ng/ml) in aqueous samples requires the injection of sample volumes up to 1000 ml. The use of an LC pump for sampling allows such volumes to be loaded easily onto a precolumn of a column-switching system. This approach has been widely applied to on-line PC-LC systems for the sensitive analysis of apolar and polar pollutants in aqueous samples [45–60]. During the enrichment step, the analytes should be well retained by the sorbent of the precolumn. Trace enrichment of rather apolar compounds has been performed on C-18 material [45,46,48,50–52,58] while carbon-based material and styrene-divinylbenzene copolymers such as PRP-1 or PLRP-S provide sufficient retention for the enrichment of relatively polar analytes [45,48,52,53,55–57]. Recently, membrane extraction disks have been used successfully for the on-line enrichment of polar pesticides from aqueous samples [59,60]. Ample experience has shown that, for most applications, rather small precolumn with dimensions of, typically, 5–10 mm \times 4.6–2 mm i.d., can be used. The small size reduces cost, enables fast sampling and prevents undue band broadening during analyte transfer to the analytical column. In most instances, precolumns are packed with 10–40 μm rather than 3–10 μm material and (thin) screens rather than (thicker) frits are used to prevent clogging during the analysis of, e.g. plasma or serum samples. It will be obvious that hydrophobic-type PC-LC is a highly efficient trace enrichment procedure, but does not contribute much to an increase in selectivity.

An interesting development is the combination of on-line dialysis and PC-LC for the automated analysis of drugs in aqueous extracts of edible products [61,62]. With this approach, an efficient separation between macromolecules and analytes is achieved while sensitivity is obtained by refocussing the analytes during dialysis on a C-18 precolumn.

Enhanced selectivity can be obtained by applying MD PC-LC. The good performance of this technique has been demonstrated for the selective trapping of various polar analytes using precolumns packed with selective sorbent materials such as cation exchangers [48,53,54], metal loaded phases [47,49] or immobilized antibodies [63,64]. An example of an experimental MD PC-LC is given in Fig. 6.5. In this study [48], selective trapping was obtained by three precolumns packed with C-18 (for non-polar analytes), PRP₁ (medium polar analytes) and a strong cation exchanger (polar basic compounds). This sophisticated MD PC-LC system has been applied for the automated and unattended determination of analytes of different polarities in aqueous samples. However, such a system is rather complex and the separated desorption of the three different precolumn fractions needs three analytical separation runs.

An imperfection of on-line trace enrichment PC-LC systems [45–60,65] is that the processing of large series of samples is still not automated because of the lack of suitable commercially available autosamplers. A modern version of the set-up shown in Fig. 6.5, improving total time of analysis and thus sample throughput by using an automated commercially available cartridge exchange system, has been described recently [65]. This system was successfully developed as a fully automated early warning system for the occurrence of 18 pollutants in various types of water at concentration levels below 5 µg/l.

Despite their potential high selectivity, MD LC-LC systems are (still) hardly applied in PRA. The major reason, solvent incompatibility, makes the interfacing of different chromatographic methods difficult and so most MD chromatography is still accomplished off-line. The first applications of MD LC-LC (late 1970s) dealt with the GPC-LC coupling.

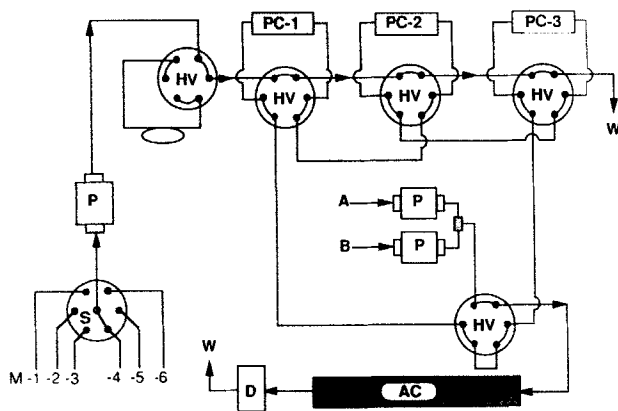


Fig. 6.5. Experimental set-up of multi-dimensional precolumn switching (MD PLC-LC) for the on-line determination of polar and apolar pollutants in waste water samples [48]. HV, high pressure valve; S, low pressure valve; M-1, sample; M-2, 10^{-3} M HClO_4 ; M-3, 50% methanol; M-4, 10^{-2} M HClO_4 ; M-5 and M-6, 10^{-3} M HClO_4 ; PC-1, $10\text{ }\mu\text{m}$ C-18 ($2 \times 4.6\text{ mm i.d.}$); PC-2, $10\text{ }\mu\text{m}$ PRP1 ($4 \times 4.6\text{ mm i.d.}$); PC-3, $13\text{ }\mu\text{m}$ A5-SCX ($4 \times 4.6\text{ mm i.d.}$); AC, $8\text{ }\mu\text{m}$ C-18 ($250 \times 4.6\text{ mm i.d.}$); P, LC-pump; A, 0.1 M potassium acetate (pH 6.0); B, methanol; D, UV photodiode array detector; W, waste.

This technique cannot be considered as a true coupled column system because the separation capacity of GPC is relatively low for the target analytes. On-line analysis of malathion in concentrated organic tomato extracts was performed with a GPC-RPLC system [66]. Using tetrahydrofuran (THF) as the mobile phase in the GPC column, fractions were directed, via the loop of an injection valve, onto an RPLC column using water/acetonitrile as the mobile phase. Because THF is a strong modifier in RPLC, only a small volume ($10\ \mu\text{l}$) could be injected from the GPC column to the RPLC column, limiting the sensitivity of the technique. An improved compatibility between GPC and RPLC was obtained using a microparticulate ($10\ \mu\text{m}$) exclusion column with an aqueous mobile phase [67], which allowed the injection of large transfer volumes (up to 2 ml; with vitamins as analytes).

MD LC-LC using two high resolution columns with orthogonal separation mechanisms was reported in 1980 [68], but only a few applications in PRA appeared afterwards.

The coupling of an aqueous RPLC system to an organic NPLC system for the separation of some dinitroaniline herbicides has been demonstrated [69]. Interfacing was established with an on-line liquid-liquid extraction of the RPLC effluent with hexane using a PTFE-membrane phase separator. However, this orthogonal system is applied to rather non-polar analytes (good GC elution behaviour) and the study only presents data obtained from standard solutions. An MD LC-LC system consisting of an anion exchanger and a C-18 column was used for the determination of two acidic herbicides in wheat to a level of about 10 ppb. Unfortunately, the supporting chromatograms do not illustrate the claimed high selectivity of the MD procedure [70].

The application of two LC separation columns with stationary phases of similar selectivities (LC-LC) also seems to be a viable approach. This technique is easy to implement and the separation power of the first column offers the possibility of performing a more efficient pre separation (clean-up) in comparison to off-line open column procedures.

In 1976, a normal phase LC-LC system was reported for the on-line clean-up and separation of organochlorine pesticides (OCPs) in milk extracts [71]. This technique was also applied for the automated sample clean-up and fractionation of OCPs and PCBs in human milk extracts [72], using the column switching assembly presented in Fig. 6.6.

Reversed phase LC-LC has been used for the determination of polar compounds in biomedical analysis [73–83], indicating the potential selectivity enhancement of this technique, which is particular favourable with non-selective UV detection at wavelengths below 240 nm [78–81].

In LC-LC, the use of samples of aqueous samples to give sufficient sensitivity is not suitable. The properties of an efficient separation column, e.g. its dimensions and small size of packing material ($<10\ \mu\text{m}$), will restrict fast sampling and increase the possibility of clogging. Furthermore, during sampling of large volumes, excessive band-broadening can be expected for the more polar analytes. Therefore, the application of LC-LC in PRA with analytes of widely different polarities will usually require a preceding concentration step.

In contrast to PC-LC, the coupled column LC is still hardly applied in PRA [84]. The possibility of performing automated and efficient clean-up of extracts of both solid and aqueous samples should, however, make it very attractive in PRA. In recent years, we have developed a number of coupled column applications for the determination of polar

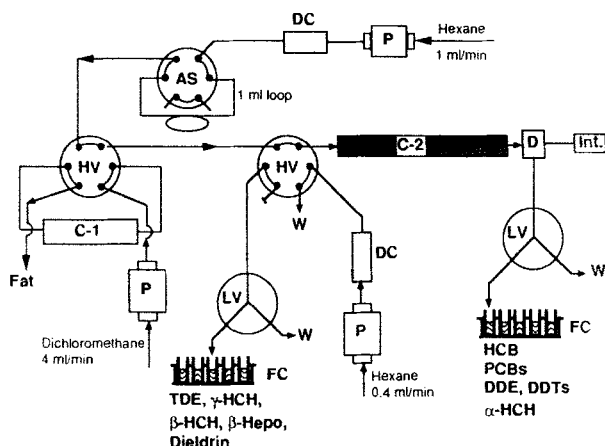


Fig. 6.6. Normal phase LC-LC system used for the fractionation of polychlorinated biphenyls (PCBs) and organochlorine pesticides [72]. HV, high pressure valve; LV, low pressure valve; P, LC-pump; AS, autosampler; C-1, 60 × 4.6 mm i.d. column packed with 10 µm Lichrosorb Si-60; C-2, 200 × 3 mm i.d. column packed with 5 µm Lichrosorb Si-60; DC, 150 × 4.6 mm i.d. drying columns filled with 63–200 µm Silica; FC, fraction collector; D, UV detector at 214 nm; W, waste.

pesticides in various matrices [85–90]. The results of these studies are discussed in the next section.

6.3. COLUMN-SWITCHING RPLC IN PESTICIDE RESIDUE ANALYSIS

6.3.1. General approach

The basic system for the column-switching procedures which has been applied [85–90] is depicted in Fig. 6.7. It includes a number of steps: after injection of a crude sample extract on the first column (C-1), a clean-up is performed with a certain volume of M-1, the mobile phase of C-1. In this step the more polar sample interferences (S1) are removed. Shortly before the first analyte starts to elute from C-1, it is switched on-line with the second column (C-2). A certain volume of M-2 (mobile phase of C-2), transfers the fraction containing the analytes (A) from C-1 to C-2. During the separation of the analytes on C-2, the first column is washed with a strong eluent (R), e.g. 100% methanol, for removal of the more apolar sample interferences (S2) and reconditioned with M-1 prior to the next injection.

The two most important parameters to be optimized in a column-switching procedure are: the choice of the first separation column (C-1), and the eluotropic strength of the clean-up solvent (M-1). It appeared from our applications [85–90] that a 15 × 3.2 mm i.d., 7 µm C-18 column (N = approx. 300) provided a sufficient pre-separation for the RPLC analysis of moderately polar pesticides in various matrices. For the selection of a proper mobile phase composition (M-1) on C-1, one must consider that a low eluotropic

strength for M-1 allows the injection of larger volumes of aqueous samples without significant band-broadening, but will restrain the effective removal of early eluting interferences (S1). A higher eluotropic strength of M-1 improves the separation between S1 and the analyte fraction (A). This is demonstrated in Fig. 6.8 which shows chromatograms of the RPLC analysis of iprodione produced by 2-ml injections of a 20-fold concentrated surface water extract which has been redissolved in acetonitrile/water (20:80, v/v). The difference in selectivity between the clean-up solvents, 20% acetonitrile (Fig. 6.8A) and pure water (Fig. 6.8B), is clearly shown. The gain in selectivity obtained by employing a well chosen eluotropic strength for M-1 to improve the clean-up was also experienced in other applications [85–90].

After the selection of C-1 and the mobile-phase composition of M-1, the final conditions can easily be determined in two experiments: after the connection of C-1 to a detector, the breakthrough volumes of the first and last eluting compound can be determined in M-1 and M-2, respectively. Figure 6.9 gives an example of such a breakthrough experiment for the analysis of some fungicides in water, showing the elution on C-1 of the first compound, 6-hydroxybentazone, with M-1, and the elution of the last compound, cyanazine, with M-2. According to these chromatograms, a volume of 3.0 ml of M-1 can be selected for clean-up on C-1 (Fig. 6.9A) and of 0.40 ml of M-2 for the transfer of all analytes from C-1 to C-2 (Fig. 6.9B).

6.3.2. Applications

An overview of column-switching methods developed at our laboratory during 1985–1990 is given in Table 6.2. For all methods mentioned in this table, an adequate clean-up for the various pesticide-matrix combinations could be obtained by using a 15×3.2 mm i.d. C-18 separation column as C-1. The different column-switching conditions applied in these methods are given in Table 6.3. The data of Table 6.2 indicate that many samples of different origins have been analysed using this approach of column-switching RPLC.

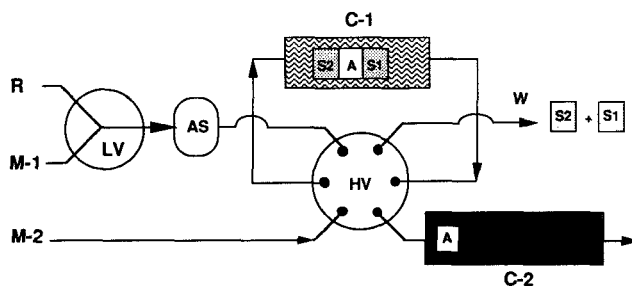


Fig. 6.7. Schematic set-up of a column-switching procedure. C-1 and C-2, first and second separation columns; AS, autosampler; S1 and S2, sample interferences; A, analyte fraction; M-1, mobile phase for clean-up on C-1 (removal of S1); R, strong eluent (removal of S2); M-2, mobile phase of C-2; LV, low pressure valve; HV, high pressure valve; W, waste.

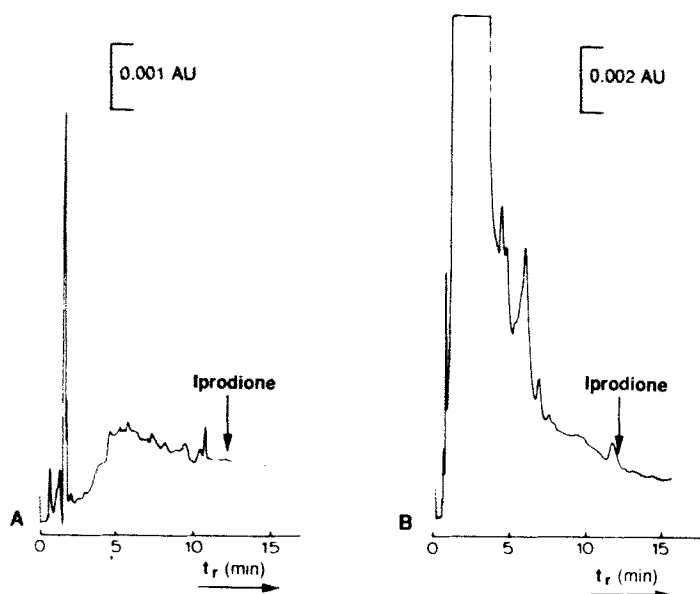


Fig. 6.8. RPLC-UV (229 nm) of iprodione in surface water using column switching with different compositions of the clean-up solvent (M-1) on C-1. (A) M-1, 5 ml of acetonitrile/water (20:80, v/v). (B) M-1, 5 ml of pure water. Injection, 2 ml of a 20-fold concentrated blank surface-water on C-1 (conditions see Table 6.2, Method 1).

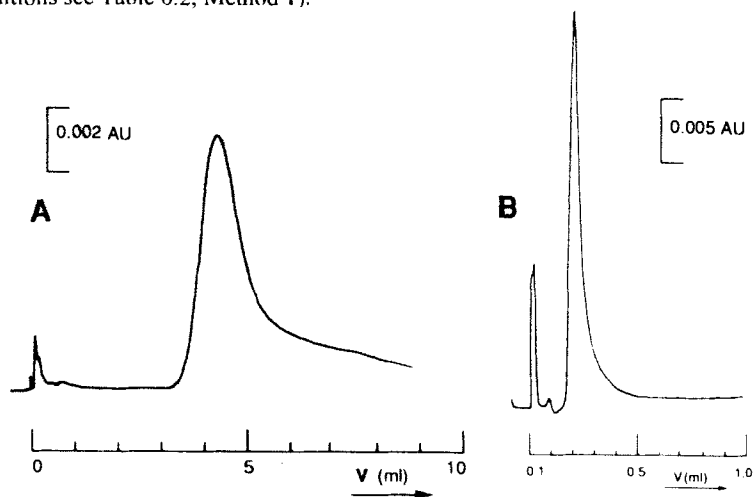


Fig. 6.9. RPLC-UV (229 nm) for selection of the column-switching conditions of Method 4 (see Table 6.2). Injection of 50 μ l of standard solutions on a 15 \times 3.2 mm i.d. column (C-1) directly connected to a UV detector. (A) Elution of first compound, 6-hydroxybentazone (192 ng), with M-1, methanol/0.03 M phosphate buffer (pH 2.7) (5:95, v/v). (B) Elution of last compound, cyanazine (49 ng), with M-2, methanol/0.03 M phosphate buffer (pH 2.7) (35:65, v/v). (Reproduced from [90] with permission of Springer-Verlag.)

6.3.3. Important features of the column-switching RPLC methods

From the column-switching procedures developed for different pesticide/matrix combinations (see Tables 6.2 and 6.3), a number of advantageous properties of the technique can be deduced. The following sections describe interesting features encountered in these studies [85–90].

6.4.1. Liquid-liquid extraction versus on-line preconcentration

On-line preconcentration procedures for the LC analysis of pesticides in rather clean aqueous samples, for which a prefiltration step is not necessary, seem favourable in circumventing a time-consuming liquid/liquid extraction. For the analysis of bromacil, diuron and the metabolite 3,4-dichloroaniline in well-water [86], we had the opportunity to compare two column-switching methods: (A) aimed at the clean-up of redissolved organic extracts of water samples; (B) involved direct sampling of a large volume of well-water onto a precolumn. In order to obtain equal concentration factors, the water sample

TABLE 6.2

OVERVIEW OF PESTICIDE RESIDUE METHODS USING COLUMN-SWITCHING RPLC

Method	Pesticide	Matrix	Extraction solvent	No. of samples	LOD ^a (ppb)	Ref.
1	Iprodione	Surface water	Dichloromethane	50	0.02	[85]
2	Bromacil, diuron, 3,4-dichloroaniline	Well water	Dichloromethane	25	0.2 0.01 0.02	[86]
3	Propoxur, carbofuran, carbaryl	Total diet	Dichloromethane	300	0.5 0.5 0.5	[87]
3	Bendiocarb, thiofanox	Beet	Dichloromethane	25	0.5 0.5	[87]
4	Bentazone	Drinking, surface and rain water	Rotavapor concen- tration	200	0.01	[88]
4	Bentazone, 6- and 8-hydroxy- bentazone, cyanazine	Maize, beans	Dichloromethane	100	20 20 20	[88]
5	Iprodione, vinclozolin, procymidone	Carrot, fennel, onion	Ethyl acetate	200	50 50 50	[89]
6	DNOC, dinoseb, dinoterb	Soil	Methanol	25	10 10 10	[90]

^aLOD = limit of detection (signal-to-noise, 3/1).

TABLE 6.3

OVERVIEW OF COLUMN-SWITCHING CONDITIONS USED FOR METHODS 1–6 (cf. TABLE 6.2)^a

Method	Sample amount per 1 ml of final extract	Injection volume (μl)	Clean-up on C-1 with M-1	Transfer of A with M-2 from C-1 to C-2
1	20 ml	2000	5 ml ACN/water (20:80)	5 ml ACN/water (47.5:52.5)
2	500 ml	100	2.5 ml MeOH/water (10:90)	0.5 ml MeOH/water (65:35)
3	5 g	1000	3.2 ml ACN/water (5:95)	0.35 ml ACN/water (35:65)
4	1.25 g	100	3.0 ml MeOH/ buffer pH 2.5 (5:95)	0.4 ml MeOH/buffer pH 2.7 (35:65)
5	1 g	150	2.0 ml ACN/water (20:80), 0.5 ml ACN/water (30:70), 0.2 ml ACN/water (40:60)	0.18 ml ACN/water (50:50)
6	20 g	75	1.1 ml MeOH/buffer pH 2.9 (23:77)	0.8 ml MeOH/buffer pH 2.9 (60:40)

^aACN, acetonitrile; MeOH, methanol; mobile phase compositions in v/v; A, analytes.

was concentrated 500 times by extraction with dichloromethane prior to the column-switching procedure (Method 2 from Table 6.3). This procedure (A) was compared with the second procedure (B) involving the direct sampling of 50 ml of the well-water onto the precolumn.

The results of these experiments, shown in Fig. 6.10, illustrate the advantage of using redissolved extracts (Fig. 6.10A), rather than the direct sampling method (Fig. 6.10B). In Fig. 6.10B, a large interfering peak shows up, making the determination of bromacil and 3,4-dichloroaniline impossible and the quantification of diuron difficult. Attempts to remove some of the polar interferences by introducing a clean-up step, with 2 ml of 10% methanol in water after preconcentration failed: the recovery of diuron decreased to 50%. It must be mentioned, however, that in contrast to on-line preconcentration, liquid-liquid extraction of aqueous samples requires a (time-consuming) manual operation.

6.4.2. Comparison between on- and off-line procedures

For the determination of propoxur, carbofuran and carbaryl (*N*-methylcarbamates) in duplicate food samples, a RPLC column-switching procedure was developed to provide automated clean-up of the extracts (Method 3) [87]. Total-diet studies require a low limit of determination in an extremely difficult matrix. Column-switching clean-up, in combi-

nation with post-column derivatization with *o*-phthalic dicarboxaldehyde (OPA) and fluorescence detection, meets the requirements of this rather complex problem. The results of the on-line procedure were compared with those of an off-line procedure which used 3-ml solid phase extraction (SPE) cartridges packed with 200 mg C-18, 40 μ m material (Baker, Deventer, The Netherlands). In order to obtain a good comparison between the two procedures, the SPE conditions were made similar to those of the on-line procedure. According to Fig. 6.7, the elutropic strength of the clean-up solvent must be adjusted so that it removes the interferences (S-1) as much as possible from the cartridge without loss of analytes (A). For desorption of the analytes, a solvent composition should be selected which restricts the elution volume (minimal dilution) but prevents breakthrough of the later-eluting interferences (S-2). After some trial-and-error experiments, the following SPE-conditions were found to be satisfactory:

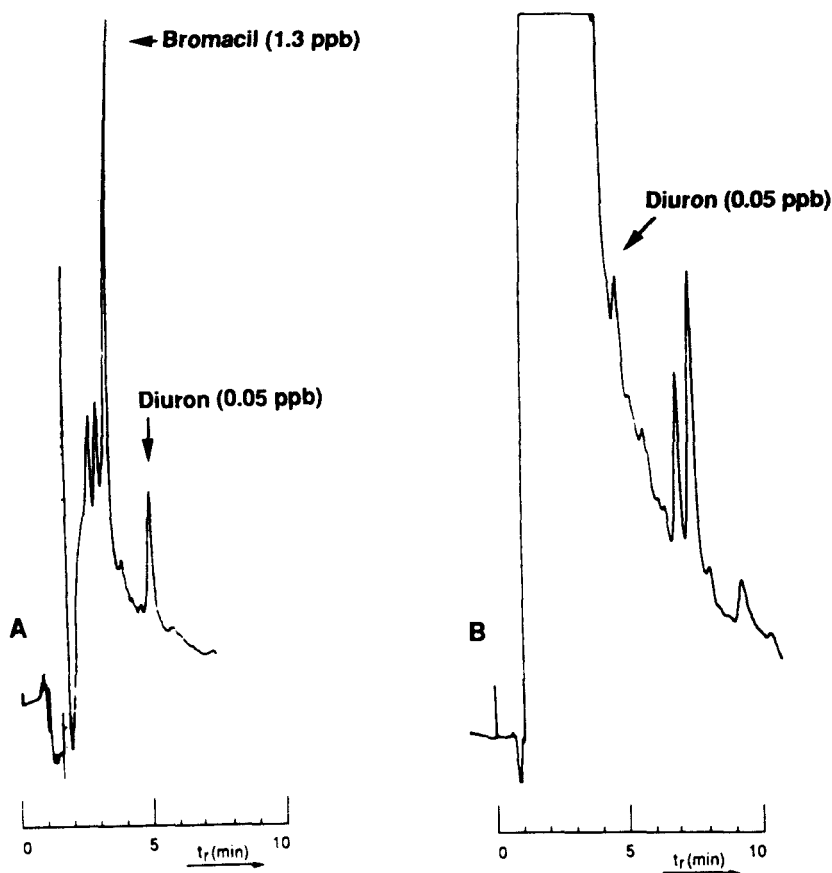


Fig. 6.10. RPLC-UV (254 nm) of a well-water sample with column switching (Table 6.3, Method 2). (A) 100 μ l injection of a 500-fold concentrated sample extract on C-1; (B) direct sampling of 50 ml of well-water onto C-1.

- preconditioning of the cartridge with 5 ml of acetonitrile followed by 10 ml of water;
- transfer of 1 ml of extract (corresponding to 10 g of total diet) onto the cartridge;
- clean-up with 5 ml of 10% acetonitrile in water;
- elution of analytes with 2 ml of 70% acetonitrile in water;
- dilution of the eluate with 2 ml of water;
- injection of 100 μ l of the final extract (corresponding to 0.25 g of total diet) onto the second separation column, (C-2), in the LC system described in ref. 87.

A 1:1 dilution of the eluate from the cartridge was necessary; without it, the resolution between propoxur/carbofuran decreased considerably ($R_s < 0.5$) owing to injection band-broadening.

A dichloromethane extract corresponding to a 250 g of blank total-diet sample was spiked with 57.5 ppb propoxur, 66.5 ppb carbofuran and 75 ppb carbaryl, as a reference material for the comparison. A simple pretreatment with acetonitrile/hexane removes most of the fatty substances [87]. The remaining residue was redissolved in 25 ml of 20% acetonitrile in water. From this solution, containing 10 g of spiked total-diet per ml, 1 ml was taken for the off-line procedure and 0.5 ml was injected onto C-1 for the on-line clean-up. The methods were compared by a fivefold analysis of the extracts. Relevant data are summarized in Table 6.4. The recoveries were from 95 to 100% for both procedures. Owing to the higher degree of automation, the repeatability of the on-line procedure is somewhat better than for the off-line procedure. The repeatability of the off-line method can be improved by using one of the recently marketed automated SPE systems such as the ASPEC (Gilson, Villiers-le-Bel, France). It was particularly interesting to see significant differences in the RSD values between standards and samples processed with SPE using one and the same cartridge for each series.

TABLE 6.4

COMPARISON OF REPEATABILITY OF ON-LINE AND OFF-LINE CLEAN-UP PROCEDURES FOR THE DETERMINATION OF THREE *N*-METHYLCARBAMATE PESTICIDES IN TOTAL-DIET EXTRACTS

Procedure ^a	Sample input	RSD (% , <i>n</i> = 5) of		
		Propoxur	Carbofuran	Carbaryl
On-line	Standards	1.8	2.0	1.8
On-line	Spiked total diet	1.4	2.0	3.3
Off-line	Standards on 1 cartridge	10	12	11
Off-line	Standards on 5 cartridges	5.7	4.2	3.2
Off-line	Spiked total diet on 1 cartridge	6.4	5.4	4.3
Off-line	Spiked total diet on 5 cartridges	6.1	3.9	4.4

^aOn-line: 1000 μ l injection on C-1 (conditions: see Table 6.3, Method 3); off-line: 100 μ l injection on a 150 \times 4.6 mm i.d. C-18 column (conditions: see text and Fig. 6.11).

As illustrated in Fig. 6.11, a drawback of the off-line method is a higher LOD caused by a 20-fold difference in dilution of the injected solution; the LOD of the off-line procedure is about 20 times higher than for the on-line procedure.

In principle, it is possible to increase the sensitivity of the SPE method by using a higher sample-load. However, to obtain the same LOD, 20 times more sample (200 g) must be brought onto the cartridge. In practice, this is very inconvenient, since the fast extraction in 10 ml tubes preceding the on-line method must then be replaced with a more time-consuming procedure using separatory funnels.

6.4.3. On-line clean-up with multi-step gradient elution

For the execution of on-line multi-step gradient elution, the low pressure three-way valve (LV) of the general set-up depicted in Fig. 6.7 is replaced by a six-way LV for the selection of different clean-up solvents (M-1) and a second high-pressure valve (HV) is installed between the autosampler (AS) and the HV used for column switching. With the additional HV, a next clean-up solvent for C-1 can be introduced (in this instance, for 3 min) by directing the flow to waste instead to C-1.

In the determination of some fungicides in fennel, it was impossible to obtain sufficient resolution between interferences (S1 and/or S2) and analytes (procymidone, iprodione and vinclozolin) using only one clean-up solvent. A similar problem was encountered in the determination of these fungicides in carrot extracts [91], with off-line SPE clean-up on C-18 cartridges. In the latter case, better results were obtained using three different sol-

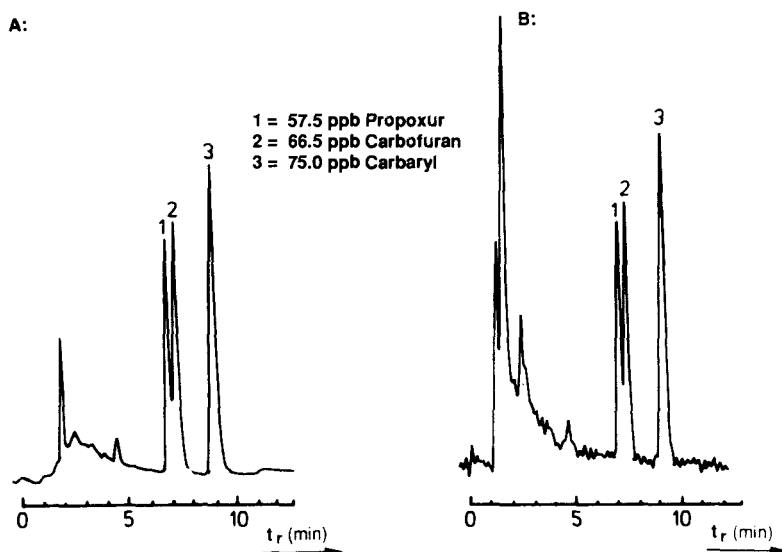


Fig. 6.11. RPLC of three *N*-methylcarbamate pesticides in total-diet extracts using two different clean-up procedures. (A) 500 μ l injection of an extract (5.0 g of total diet) cleaned on-line with column-switching; (B) 100 μ l injection of an extract (0.25 g of total diet) after off-line clean-up; fluorescence detection with $\lambda_{ex} = 340$ nm and $\lambda_{em} = 455$ nm (Table 6.3, Method 3).

vents of increasing eluotropic strength for clean-up. The conditions used in this off-line procedure were extrapolated to an on-line procedure; here, also, a three-step gradient elution on C-1 was found to yield at least partial separation between analytes and interferences (Method 5, Table 6.3). In this case, however, we had to compromise between an efficient clean-up and good recovery levels.

The first eluting compound, procymidone, breaks through from C-1 before transfer during the last clean-up step with 40% acetonitrile lowering the recovery to 50%. The last eluting compound, vinclozolin, determines the volume of the transfer solvent, M-2 (50% acetonitrile). Vinclozolin can be transferred completely from C-1 to C-2 with 0.2 ml of 50% acetonitrile. However, it appeared that with fennel and carrot extracts, many interferences still co-elute using this transfer volume. Therefore, a transfer volume of 0.18 ml of 50% acetonitrile was used, yielding a recovery for vinclozolin of 94%. A result of this step-gradient elution clean-up is shown in Fig. 6.12; the increase in selectivity from a two- to a three-step gradient clean-up is clear. In spite of the concessions made for the recoveries of procymidone and vinclozolin, the reproducibility for fennel extracts spiked at levels between 0.2 and 2.4 mg/kg (ppm) gave favourable relative standard deviations ($n = 6$) for iprodione, procymidone and vinclozolin of 4, 5 and 4%, respectively. The performance of

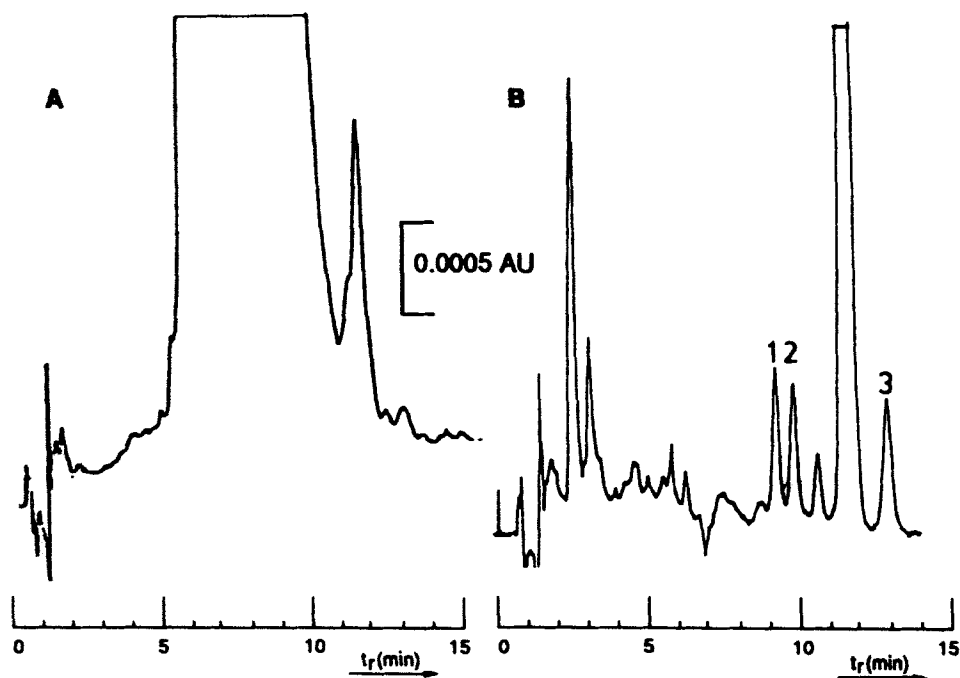


Fig. 6.12. RPLC-UV (229 nm) of three fungicides in fennel extracts using step-gradient elution for the on-line clean-up with column switching (Table 6.3, Method 5). Injection of 150 μ l of extract (75 mg fennel) on C-1; ACN, analyte transfer with 0.18 ml of 50% acetonitrile (ACN). (A) Clean-up with a one-step gradient elution on C-1: 2 ml of 20% ACN and 0.40 ml of 40% ACN; (B) clean-up with a two-step gradient elution on C-1: 2 ml of 20% ACN, 0.5 ml of 30% ACN and 0.20 ml of 40% ACN. Peaks: 1. 0.16 ppm iprodione; 2, 0.22 ppm procymidone; 3, 0.24 ppm vinclozolin.

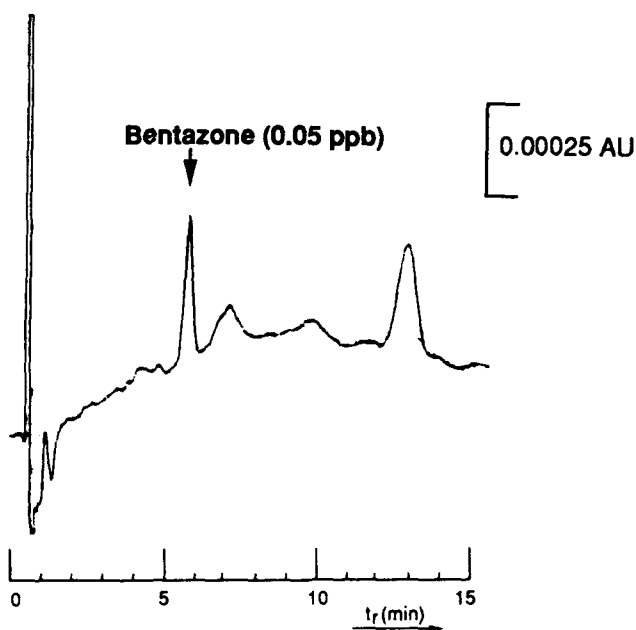


Fig. 6.13. RPLC-UV (229 nm) of bentazone in rain-water (Table 6.3, Method 4). Injection of 100 μ l of extract (corresponding to 100 ml of rain water) on C-1. Column-switching conditions: clean-up with 4.0 ml of MeOH/buffer (pH 2.5) (10:90), transfer with 0.25 ml of MeOH/buffer (pH 3.2) (35:65).

the system remained constant during 2 months of intensive use. However, it must be admitted that establishing the rather complex clean-up conditions required a lot of experimental work.

6.4.4. Improvement of sensitivity and selectivity

In LC-LC, the (pre)separation between analytes and interferences is performed on an efficient separation column. In an ideal situation, a concentrated extract should be cleaned on C-1 in such a way that only the analytes are transferred to C-2. This may be realized with very specific clean-up techniques, such as immuno-affinity chromatography, focused on limited numbers of compounds [63,64]. However, with RPLC retention as the criterion for selectivity, part of the (abundant) sample interferences will always be transferred together with the analytes. Hence, the success of a procedure is determined by the effectiveness of the clean-up with M-1, used for the removal of S1 and the volume of the analyte fraction (A) of M-2 which prevents the transfer of S2. The latter volume is determined by the elution time of the last compound (see Fig. 6.9). This means that, if one is using a certain C-1, the selectivity is related to the retention range of the compounds of interest. A decrease in the retention (polarity) range of the analytes involved will result in increased selectivity. Consequently, one might expect optimal selectivity in a situation where only one compound is analysed. Here, the selectivity, and thus the sensitivity in

PRA, can be enhanced considerably by using very narrow clean-up/transfer conditions. An example is presented in Fig. 6.13, which shows the determination of bentazone in rain-water at a level of 0.05 ppb. With the application of a simple acid/base liquid-liquid sample pretreatment [88] and adjustment of the optimal column-switching conditions (Method 4, Table 6.3), bentazone was determined in rain- and surface-water down to 0.01 ppb.

Because of the clean nature of drinking-water, one would not expect column-switching to be necessary for the clean-up of extracts prior to LC analysis. However, column-switching is also useful for the determination of bentazone in drinking-water. This is illustrated in Fig. 6.14, which shows clearly that the absence of a clean-up step can easily lead to errors in the identification and quantification of bentazone. In the situation depicted in Fig. 6.14A, false positives can easily be discriminated.

6.4.5. Additional advantages of column-switching

An effective function of column-switching is the protection of the main separation column (C-2) from clogging by solid particles from the sample. Prefiltration over a $0.45\ \mu\text{m}$ filter is not always appropriate in pesticide residue analysis. As an example, filtration of redissolved vegetable extracts prior to RPLC-analysis of fungicides results in a considerable loss of analytes [89]. A layer of lipophilic precipitate probably acts as a sorbent during filtration.

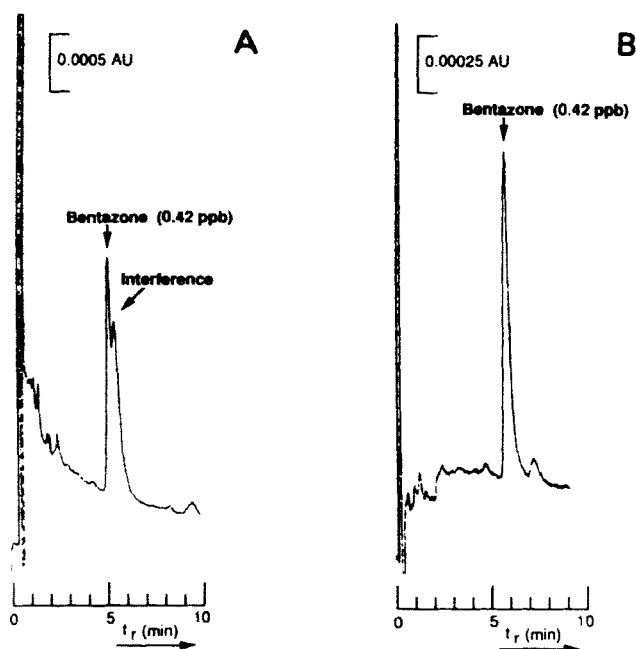


Fig. 6.14. RPLC of a drinking-water sample without (A) and with (B) the use of an on-line column-switching technique. Injection of $50\ \mu\text{l}$ of extract corresponding to 16.6 ml of water; conditions as in Fig. 6.13.

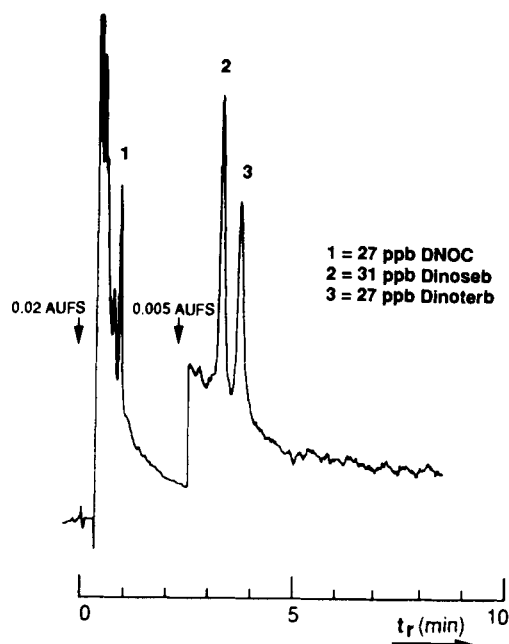


Fig. 6.15. RPLC-UV (365 nm) of dinitrophenols in soil extracts using on-line clean-up with column switching (Table 6.3, Method 6). Injection of 75 μ l of extract (corresponding to 20 g/ml) on C-1. (Reproduced from [90] with permission of Springer-Verlag.)

Column switching was also needed for the RPLC determination of three dinitrophenols in sediment, at a level of 10 ppb. Using the conditions of Method 6 (Table 6.3), the fraction containing the dinitrophenols was transferred to C-2 and the compounds detected with UV at 365 nm. The chromatogram of a spiked blank soil sample is shown in Fig. 6.15.

In this case, column switching is not essential to increase selectivity, since almost the same chromatogram can be obtained without it. The guard function of C-1 is essential in this application; filtration of a redissolved spiked soil extract results in total loss of the analytes. Therefore injection of unfiltered extracts in the LC-system is unavoidable.

However, if one removes column switching, the main 3- μ m C-18 separation column (C-2) is clogged after a few injections and cannot be unclogged by flushing it with a strong eluent (100% acetonitrile).

In the final procedure, the dinitrophenols were extracted (1 h ultrasonic) from soil (20 g) with alkaline methanol (50 ml). After filtration, 200 ml of water and 10 ml of hydrochloric acid were added to the methanol filtrate. The aqueous phase was extracted three times with dichloromethane (50, 25 and 25 ml) and the collected organic phases were dried over sodium sulphate and concentrated in a Kuderna-Danish apparatus. The remaining dichloromethane was evaporated to dryness with a gentle stream of nitrogen and the residue redissolved in 200 μ l of acetonitrile followed by the addition of 800 μ l of water before injection.

For the analysis of rather clean sample extracts, for which column switching is not absolutely necessary to prevent clogging or to improve the selectivity and/or sensitivity, it is tempting to circumvent the procedure. However, in several cases, the use of column switching is beneficial in reducing the time of analysis, since no later eluting matrix peaks (S2) are transferred to the second separation.

6.5. RECENT DEVELOPMENTS IN COUPLED-COLUMN RPLC

The applications of reversed phase LC-LC [85–90] to various pesticide/matrix combinations discussed above, clearly demonstrate the usefulness of this technique in PRA. In contrast to off-line procedures, it provides a fully automated clean-up of extracts and considerable enhancement in sensitivity and selectivity.

Suitable coupled-column conditions are, however, still found by trial-and-error optimization, in line with the present state of the art in biomedical applications [73–83]. This situation makes the search for proper on-line conditions highly dependent on the experience of the analytical chemist, and very complex and tedious if a number of different clean-up steps are necessary to obtain sufficient clean-up [89].

Selectivity in the analysis of single polar compounds is an important feature of coupled-column RPLC. This was demonstrated for the analysis of bentazone in aqueous samples [88]. In this work, 100 μ l of a manually prepared concentrated extract was injected onto a short first, C-18 column. The rather small injection volume and subsequent low transfer volume provided a superior selectivity. Sampling of larger volumes on such a short separation column limits the possibility of performing an efficient preseparation of the analyte from the early eluting sample interferences. This effect is shown in Fig. 6.10 for the determination of diuron in well-water [86].

One might expect improvement in the selectivity by applying longer C-1 columns with higher separation power. It is therefore interesting to investigate the potential of coupled column RPLC for trace analysis of a single polar pesticide in aqueous samples, using direct large-volume injection.

The next two sections present more recent results in which the coupled-column method has been used to develop procedures for (i) the direct assay of (single) polar pesticides in aqueous samples (SRMs) and (ii) the simultaneous determination of a number of polar pesticides having a large range in retention (MRMs), involving more rational optimization procedures for establishing proper coupled-column conditions.

6.5.1. Single residue method development strategy

An important advantage of the application of RPLC to aqueous samples is that the low elutropic strength of water allows the injection of relatively large sample volumes without extensive band-broadening. This provides an efficient on-line trace-enrichment. However, problems are encountered in using this approach for highly polar analytes. Retention is small, even on highly hydrophobic C-18 bonded silica phases and, therefore, trace enrichment becomes difficult because the analyte is already eluting during the injection. Secondly, only a limited separation capacity is available to separate the analyte(s)

from the excess of early eluting and UV-absorbing interferences, such as anions, and humic and fulvic acids, which are always present in environmental samples. The parameters that primarily determine the sensitivity and selectivity of a method of column-switching RPLC are summarised in Fig. 6.16.

Based on experience [92,93], a rational approach to column-switching RPLC method development has been derived [94]. This approach, schematically shown in Fig. 6.17, involves the application of a number of steps which provide information on the important parameters (see Fig. 6.16) which, in turn, will determine the possibility of a coupled-column procedure.

Along the lines of Fig. 6.17, RPLC column-switching procedures were developed for the assay of very polar analytes, namely chloroallyl alcohol [92], ethylenethiourea [93] and methylisothiocyanate [94] at a level of $1\text{ }\mu\text{g/l}$ in water samples, by direct large-volume injection. The relevant properties (capacity factor, detection wavelength and molar extinction coefficient) of the highly polar analytes and the established column-switching conditions are given in Table 6.5. The results of the procedures developed are presented in Fig. 6.18, which clearly shows the distinct increase in selectivity achieved using coupled-column RPLC.

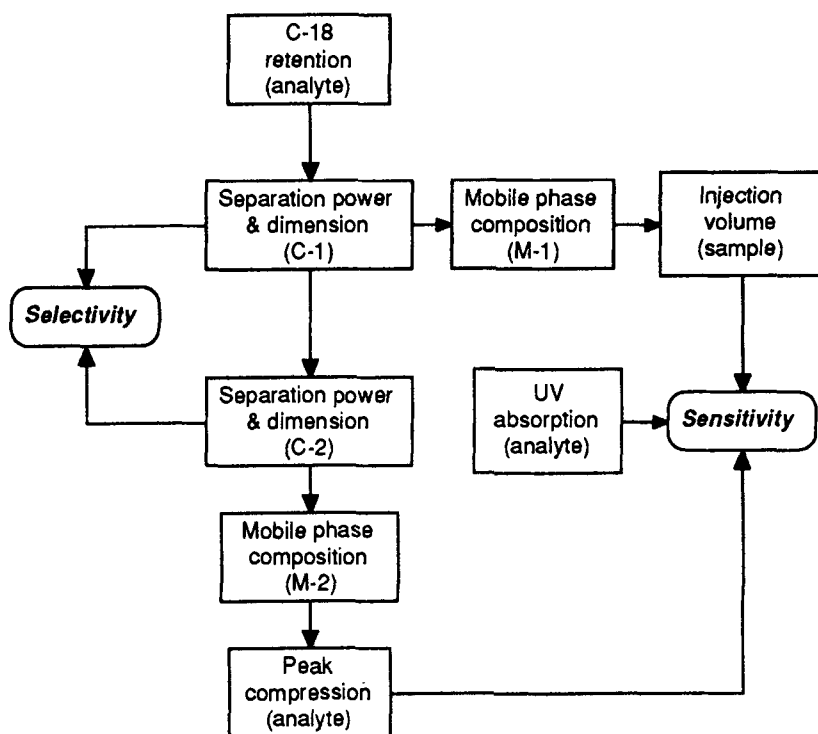


Fig. 6.16. Column-switching RPLC parameters influencing sensitivity and selectivity. C-1 and C-2, first and second separation column; M-1 and M-2, mobile phase of C-1 and C-2, respectively.

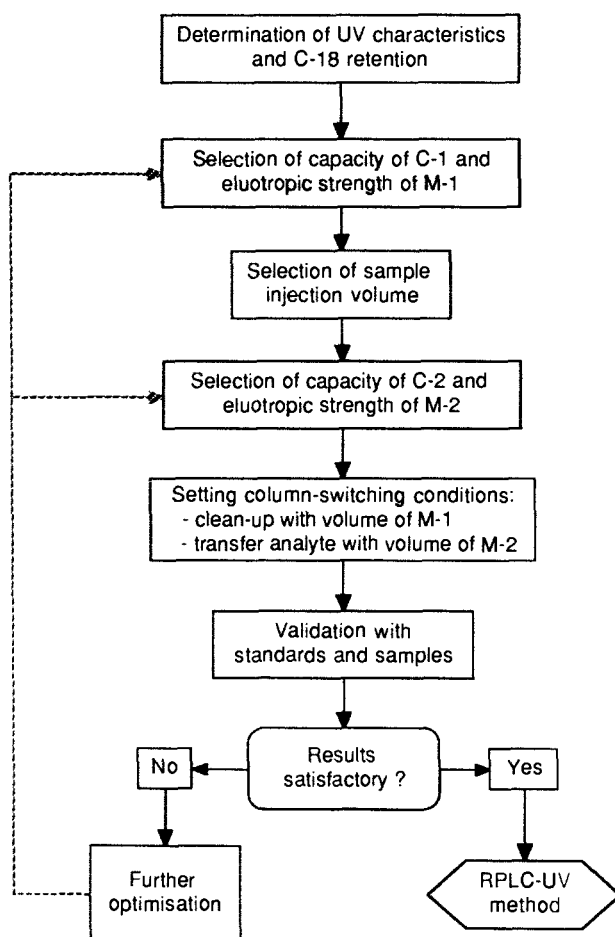


Fig. 6.17. Scheme of column-switching RPLC method development for single analytes.

As can be deduced from the data presented in Table 6.6, all three analytes possess one less than optimal parameter for their determination by RPLC-UV. The C-18 retention of ETU is very poor, the UV detection wavelength of CAAL is non-selective and MITC has a low molar extinction coefficient. Nevertheless, direct analysis of aqueous samples for these analytes yields an LOD of $1 \mu\text{g/l}$, indicating that one can handle one unfavourable property and still obtain remarkable sensitivity. By the use of a relatively simple manual concentration step, the LODs can be lowered to the EC drinking-water limit of $0.1 \mu\text{g/l}$ [92–94].

Even better results are to be expected for analytes which have more favourable properties of retention and detectability. Therefore the potential of coupled-column RPLC was investigated for the development of methods employing direct sample injection combined with high sample throughput (analysers) to reach the EC limit of $0.1 \mu\text{g/l}$. Indeed, bentazone and isoproturon can be assayed within 10 min with direct injection of the sample at a

level of 0.1 $\mu\text{g/l}$ [95]. Method development becomes straightforward, using the scheme in Fig. 6.17 and the considerations given in [95]. Information on relevant properties of the analytes and established column-switching conditions are given in Table 6.6, which includes information on isoproturon and bentazone and on methabenzthiazuron, for which a coupled-column method was recently developed. The high molar extinction coefficients and, even more importantly, the relatively high C-18 retention, provides sufficient sensitivity, while the application of small transfer volumes yields the required selectivity (see also Fig. 6.16).

Examples of the rapid RPLC-UV analysis of pesticides in environmental water samples at the sub-ppb level are given in Fig. 6.19. The coupled-column procedures, which

TABLE 6.5

DETAILS OF HIGHLY POLAR COMPOUNDS ANALYSED BY MEANS OF LARGE-VOLUME INJECTION COLUMN-SWITCHING RPLC WITH UV DETECTION

Parameter	Ethylenethiourea (ETU)	Chloroallyl alcohol (CAAL)	Methylisothiocyanale (MITC)
Formula	$\begin{array}{c} \text{CH}_2 - \text{NH} \\ \quad \diagdown \\ \text{CH}_2 - \text{NH} \quad \text{C} = \text{S} \end{array}$	$\begin{array}{c} \text{Cl} \quad \text{CH}_2\text{OH} \\ \diagdown \quad \diagup \\ \text{C} = \text{C} \\ \diagup \quad \diagdown \\ \text{H} \quad \text{H} \end{array}$	$\text{CH}_3 - \text{N} = \text{C} = \text{S}$
Water soluble (g/l)	20	Infinite	8
k^a	1.6	7.0	20
λ (nm)	233	205	237
ε (l/mol cm)	18 000	10 000	3000
C-1, $L \times \text{i.d.}$ (mm)	150 \times 4.6	50 \times 3	50 \times 4.6
Material	Hypersil ODS, 5 μm	Chromspher C-18, 5 μm	Microspher C-18, 3 μm
C-2, $L \times \text{i.d.}$ (mm)	150 \times 4.6	100 \times 4.6	100 \times 4.6
Material	Hypersil ODS, 5 μm	Microspher C-18, 3 μm	Microspher C-18, 3 μm
M-1	ACN/water ^b (1:99)	Water	ACN/water (40:60)
M-2	ACN/water ^b (1:99)	MeOH/water (5:95)	ACN/water (50:50)
Clean-up volume (M-1) (ml)	2.6	1.2	1.9
Transfer volume (M-2) (ml)	0.44	0.80	0.40
Sample vol. (μl)	200	200	770
Time of analysis (min)	5	7	7
LOD ^c ($\mu\text{g/l}$)	1	1	1

^aOn 5 μm Hypersil ODS; mobile phase, pure water for ETU/CAAL/MITC.

^bContaining 0.2% of ammonia.

^cDetection limit with direct sample injection.

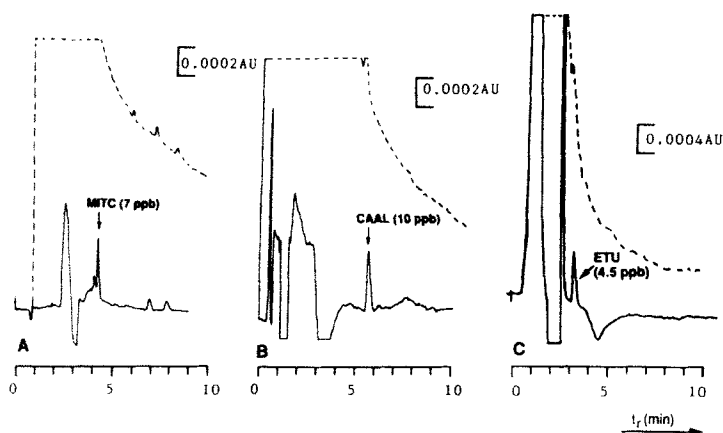


Fig. 6.18. Coupled-column RPLC with direct sample injection of environmental aqueous samples spiked with highly polar analytes. (A) Surface water; (B and C) ground-water. RPLC conditions (see Table 6.5). Dashed lines, chromatograms obtained using two columns connected in series without column switching (mobile phase M-2).

were developed within a few days, showed linear ranges over more than three decades for the three pesticides listed in Table 6.7. The reproducibility of the methods, checked with spiked environmental water samples at levels from 0.1 to 1.0 $\mu\text{g/l}$ ($n = 5$ for each level), was better than $\pm 7\%$. Several hundreds of samples of ground-, surface-, rain-, and drinking-water were investigated using the coupled-column methods described above.

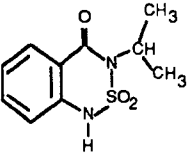
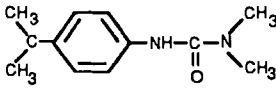
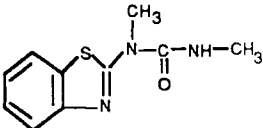
6.5.2. Multi-residue method development strategy

The SRM development strategy discussed above makes essential use of small transfer volumes to achieve highly selective and sensitive methods of analysis for the determination of single polar pesticides. In multi-residue analysis, dealing with analytes of significantly different polarities, the application of small transfer volumes will be unlikely. In addition to this important feature, the MRM methodology presents two essential difficulties compared to the SRM strategy mentioned above. First, PRA deals with various pesticide/matrix combinations (the multi-matrix aspect, see Section 6.1) and, secondly, analytes with significantly different properties will also differ in UV detectability, making the direct sampling approach as applied for aqueous samples, less attractive. Thus for a generally applicable multi-residue methodology, it is convenient to perform extraction/concentration using an organic solvent, because this will have broad application to both solid and liquid samples.

Despite the necessity of using larger transfer volumes, coupled-column RPLC is still an attractive and flexible technique for the multi-residue analysis. The major advantages of the coupled-column technique are that it can be automated and can perform an efficient separation between the excess of early eluting co-extracted sample constituents and the first-eluting analyte of interest.

TABLE 6.6

DETAILS OF POLAR PESTICIDES ANALYSED BY MEANS OF LARGE-VOLUME INJECTION COLUMN-SWITCHING RPLC WITH UV DETECTION. C-1 (50 × 4.6 mm i.d.) AND C-2 (100 × 4.6 mm i.d.) ARE PACKED WITH 3 µm MICROSPHER C-18

Parameter	Bentazone	Isoproturon	Methabenzthiazuron
Formula			
Water soluble (mg/l)	500	70	59
k^a	>100	>100	>100
λ (nm)	220	240	267
ϵ (l/mol cm)	25 000	22 000	14 000
M-1	MeOH/buffer ^b , pH 2.3 (50:50)	ACN/water (47.5:52.5)	ACN/water (42:58)
M-2	MeOH/buffer ^b , pH 2.7 (50:50)	ACN/water (47.5:52.5)	ACN/water (45:55)
Clean-up volume (M-1) (ml)	4.65	5.85	5.90
Transfer volume (M-2) (ml)	0.50	0.45	0.45
Sample vol. (µl)	2000	4000	4000
Time of analysis (min)	10	8	8
LOD ^c (µg/l)	0.1	0.1	0.1

^aOn 5 µm Hypersil ODS; mobile phase, pure water for isoproturon and methabenzthiazuron and 0.1% phosphoric acid for bentazone.

^b0.1% phosphoric acid.

^cDetection limit with direct sample injection.

A straightforward approach to MRM development using coupled-column RPLC was made for an inventory of pesticides presently registered in The Netherlands for use on cereals [96]. As listed in Table 6.7, the target group of pesticides contains neutral, acidic and basic analytes. Obviously, it will be difficult for LC to separate these compounds efficiently, or to provide sufficient selectivity between matrix interferences and the analytes in a single run. Suitable conditions for on-line clean-up and separation were found using a careful interpretation of the retention behaviours of the pesticides, expressed as the plots of $\ln k$ versus ϕ (k is the capacity factor of analyte and ϕ is the fraction of organic modi-

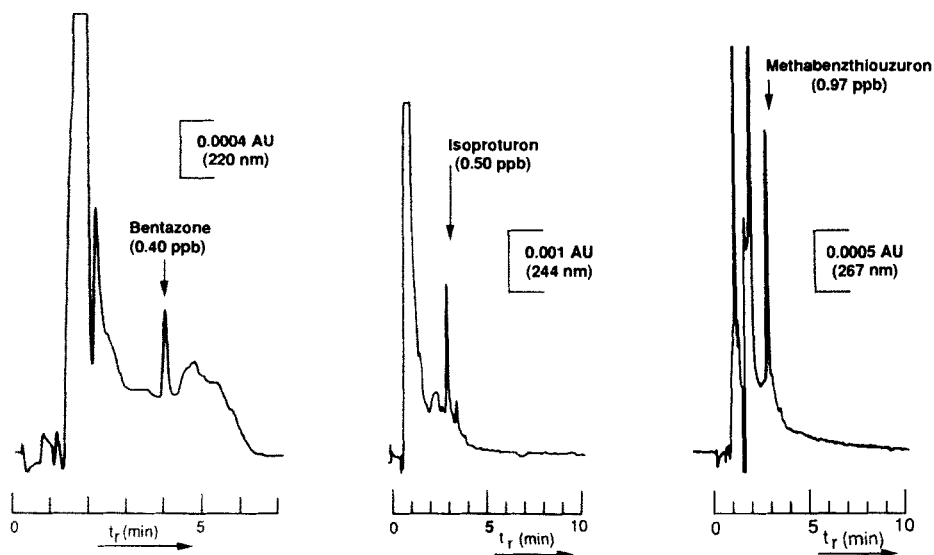


Fig. 6.19. Coupled-column RPLC with direct sample injection of surface-water samples spiked with polar pesticides. RPLC conditions (see Table 6.6).

fier). These plots are constructed using the data obtained from different isocratic experiments.

For sensitivity of the (UV) detection, it appeared that step-gradient elution, applied with the LC-system depicted in Fig. 6.20 was superior to linear-gradient elution because of the reduced base-line drift [96]. A typical chromatogram obtained by the procedure using programmed UV detection is shown in Fig. 6.21.

The procedure applied was based on a visual interpretation of the $\ln k$ versus ϕ plots [96] and remains, to a certain extent, arbitrary with regard to the selection of column-switching conditions, requiring additional experimental work of suitable conditions.

A computer program has been developed to find suitable column-switching conditions for complex separations more rapidly [97]. The underlying diffusion model for this program uses experimentally determined $\ln k$ versus ϕ relationships, and accurately predicts retention times and peak volumes of analytes eluting under the step-gradient conditions applied in coupled-column RPLC. The application of the optimization procedure is schematically presented in Fig. 6.22.

The first step is to enter into the computer program an input file holding the values of the parameters which will serve as constants for a particular application. These are the a , b and c coefficients of the quadratic $\ln k$ versus ϕ relationships for the compounds to be separated and the constants of the LC system. The relevant parameters are the injection volume, the gradient delay time (t_d), the time constant (τ) for the distortion of the solvent front, the flow and the efficiency of the column (H). The second step is to enter the variables for an elution program. The program asks for the number of steps and the length (t , min) and fraction of modifier (ϕ) of each step. After the simulation, the analyst must

TABLE 6.7

STRUCTURAL FORMULAE AND UV CHARACTERISTICS OF THE PESTICIDES TO BE DETERMINED IN CEREALS

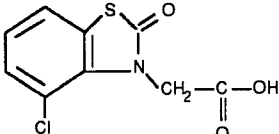
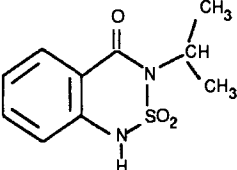
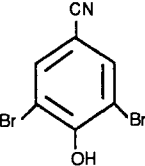
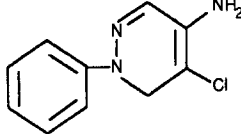
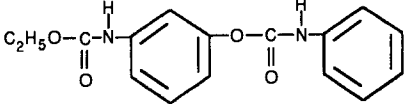
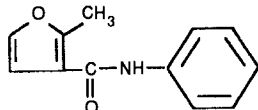
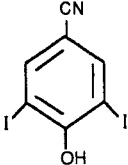
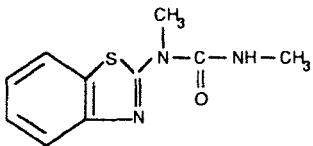
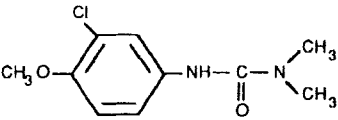
Pesticide (l/mol cm)	Formula	λ_{\max} (nm)	ϵ_{\max} (l/mol cm)
Benazolin		217	35 000
Bentazone		217	25 000
Bromoxynil		217	30 000
Chloridazon		229	27 000
Desmedipham		233	35 000
Fenfuram		258	19 000
Ioxynil		233	35 000

TABLE 6.7 (continued)

Pesticide (l/mol cm)	Formula	λ_{\max} (nm)	ϵ_{\max} (l/mol cm)
Methabenzthiazuron		217	26 000
Metoxuron		240	16 000

decide from the calculated resolutions (R_s), whether other elution conditions must be tried out to improve the separation. If the resolution seems appropriate, the calculated intensity and peak volume data are converted into a graphical representation of the chromatogram which includes the exact volumes needed for clean-up and transfer in the column-switching procedure.

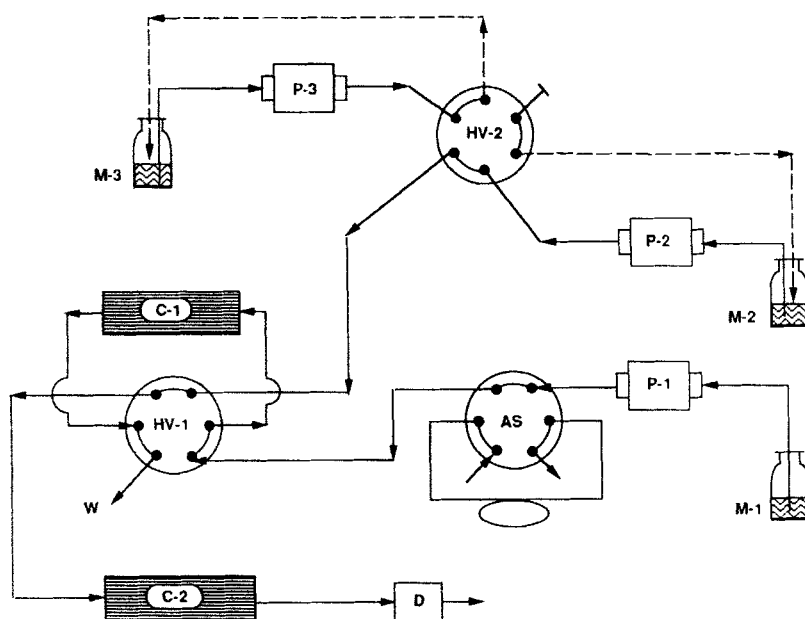


Fig. 6.20. Scheme of a coupled-column RPLC system including the ability to perform one-step gradient elution. AS, autosampler; HV-1 and HV-2, high pressure switching valves; P-1, P-2 and P-3, LC pumps; M-1, M-2 and M-3, mobile phases; C-1 and C-2, first and second separation column; D, UV detector; W, waste.

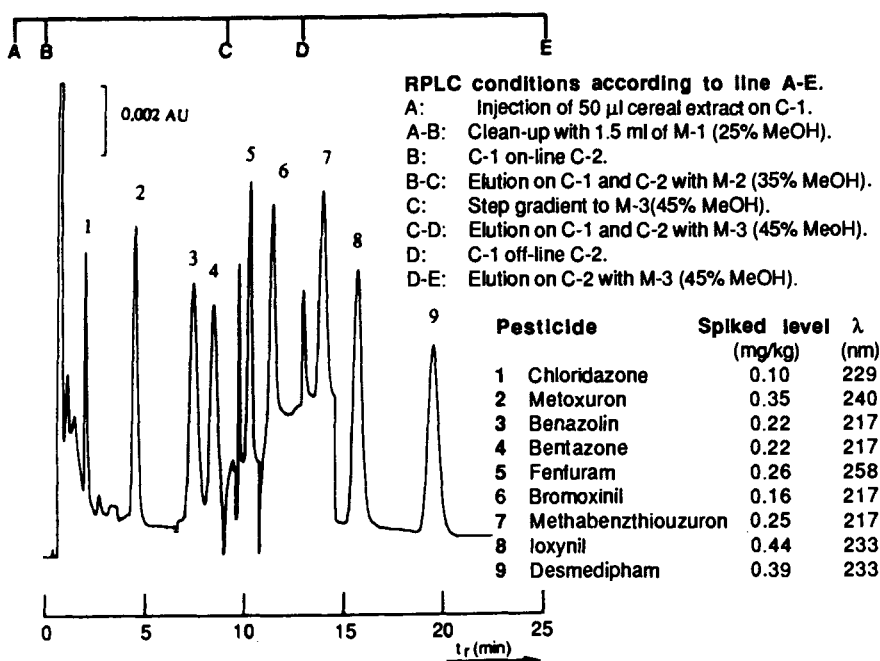


Fig. 6.21. RPLC analysis of 9 pesticides spiked to a cereal extract (2.5 g/ml) using column switching, step-gradient elution and variable UV wavelength detection. M-1, M-2 and M-3, mobile phases (see also Fig. 6.20) of 25, 35 and 45% of methanol (v/v), respectively, in a 0.025 M sodium acetate/0.03 M phosphoric acid buffer (pH 2.7); flows are set at 1 ml/min. C-1, 50 \times 3 mm i.d., 5 μ m C-18; C-2, 100 \times 3 mm i.d., 5 μ m C-18.

The viability of this approach was tested on the residue-analysis of procymidone and iprodione in fennel extracts. An earlier column-switching method developed for this [89] included a rather complicated multi-step gradient elution on a 15 \times 3.2 mm i.d. C-18 column to obtain an effective clean-up (see also Fig. 6.12). In the current study, a 5- μ m C-18, 50 \times 3 mm i.d. column was selected as the first column (C-1). After input into the computer program of the a , b and c coefficients of the $\ln k$ versus ϕ plots, this particular separation problem could be resolved easily by computer simulation. Figure 6.23 shows clearly that efficient separation between the analytes and the major interference peak on the first C-18 column requires the application of a clean-up solvent with a high elutropic strength ($\phi > 0.6$). Such a mobile phase allows elution of the analytes from the first to the second column but prevents elution of the interference. In such a case, the application of a column-switching clean-up by means of heart-cutting seems favourable. By selecting a 100 \times 4.6 mm i.d. column packed with 3- μ m Microspher C-18 as the second separation column (C-2), and mobile phases (M-1 and M-2) of methanol/water (70:30) suitable column-switching conditions were rapidly established using the computer simulation. The experimental result of the clean-up procedure with the coupled-column method, a clean-up volume of 0.85 ml of M-1 and a transfer volume of 0.30 ml of M-2, is given in Fig. 6.24.

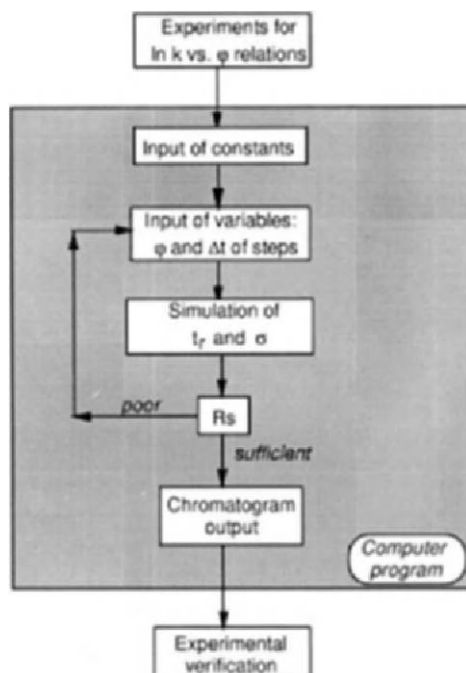


Fig. 6.22. Scheme of the step-gradient elution optimization procedure.

Compared to the trial-and-error optimization procedure [89], the method development using chromatogram simulation yields a significant time saving in finding proper clean-up and separation conditions, with benefits in reducing the solvent consumption and extending the column lifetime.

Initially, the simulation program was developed to establish proper clean-up conditions and was therefore focused on the elution of compounds on the first column of the coupled system. However, the program can predict the elution of analytes on both columns if they use the same packing material. Consequently, the computer program can be applied as a powerful tool in multi-residue analysis and for optimizing conditions for on-line clean-up of extracts using column-switching and subsequent step-gradient elution for efficient separation.

At present work is underway [98] to use this approach in describing a generally applicable strategy for developing coupled-column RPLC methods for groups of polar pesticides in environmental water samples, which is needed in flexible pesticide monitoring programs. The strategy was tested for the determination of a remaining group of polar pesticides as part of a ground-water survey to monitor about 90 pesticides. A single extraction method, liquid/liquid extraction with dichloromethane, was chosen for isolating and concentrating the pesticides. Organophosphorus compounds, triazine herbicides and *N*-methylcarbamates could be determined in the same samples.

The pesticides involved in this study are listed in Table 6.8. They have different polarities and so the search for efficient separation conditions will be difficult. To achieve

clean-up by means of column-switching, and to obtain sensitive and selective detection, it is essential that three criteria are met:

- I. The first-eluting analyte must have a retention which is at least twice as large as that of the unretained compound (clean-up in first part of the chromatogram).
- II. The resolution (R_s) between two adjacent peaks must be at least 1.2 to prevent problems due to UV wavelength switches and/or changes in the mobile phase compositions.
- III. The total time of the chromatographic run should be kept relatively short (for sensitivity and sample throughput).

Based on preliminary experiments, a 100×4.6 mm column packed with $3 \mu\text{m}$ Microspher C-18 was selected, with mobile phases of methanol/0.03 M phosphate buffer (pH 3.2) for a proper elution of the pesticides.

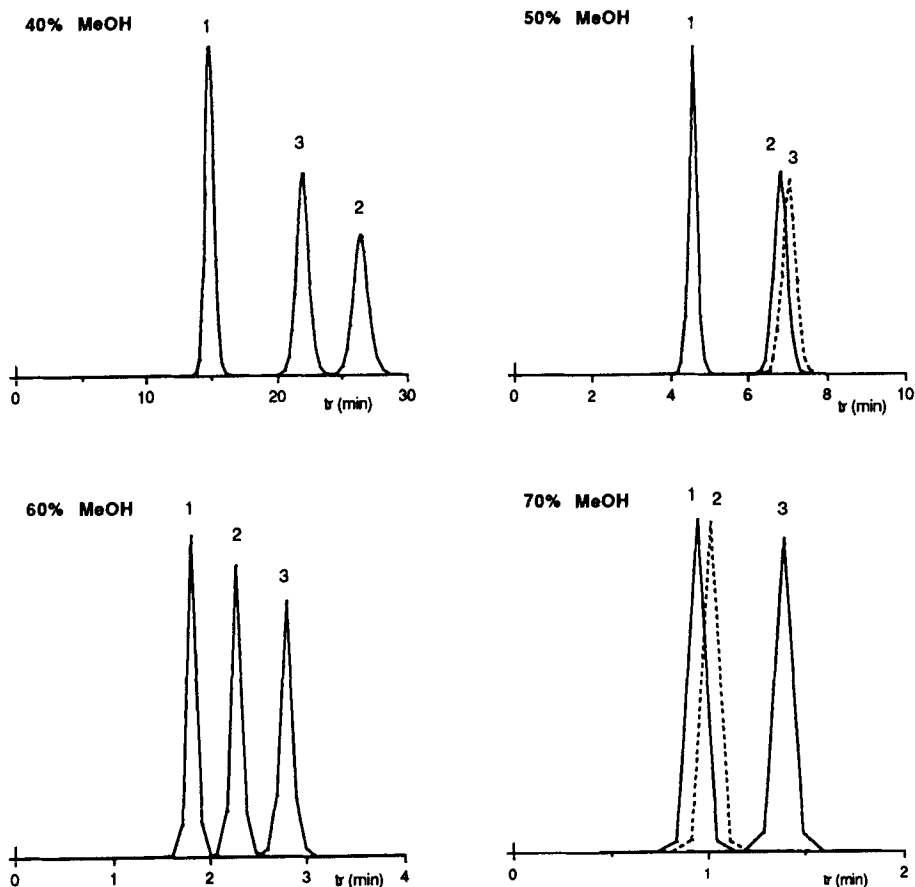


Fig. 6.23. Chromatogram simulation of the RPLC analysis of procymidon (1), iprodione (2) and a fennel interference (3) on a $5\text{-}\mu\text{m}$ C-18 column (50×3 mm i.d.) using mobile phases of methanol (MeOH)/water.

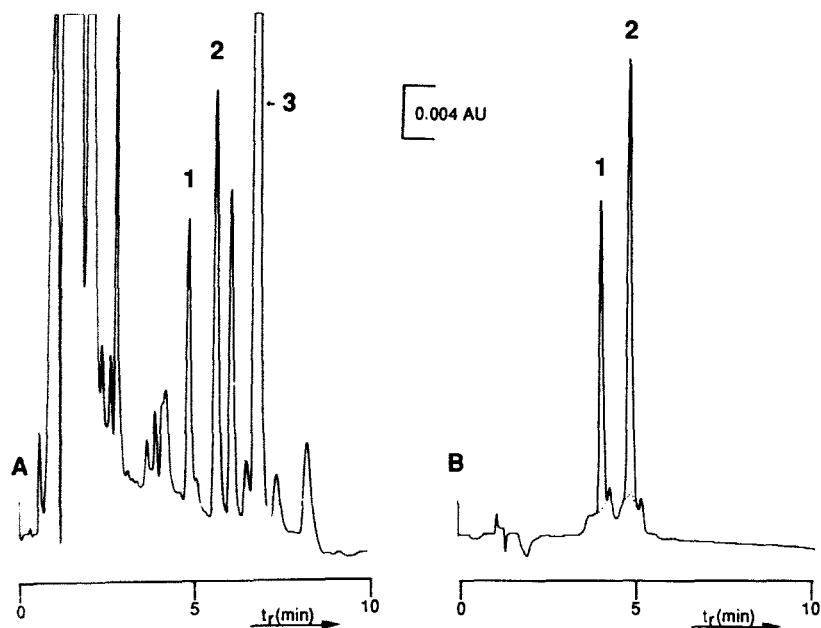


Fig. 6.24. Coupled-column RPLC-UV of a fennel extract (1 g/ml) containing 1.0 ppm of procymidone (1), 1.3 ppm of iprodione (2) and a fennel interference (3). UV detection at 229 nm. Injection: 50 μ l on C-1 (50 \times 3 mm i.d., 5 μ m C-18); C-2, 100 \times 4.6 mm i.d., 3 μ m C-18. Mobile phases (M-1 and M-2), methanol/water (70:30, v/v) set at 1 ml/min. (A) Chromatogram obtained using two columns connected in series without column switching (mobile phase M-2). (B) Application of column-switching conditions obtained with simulation: clean-up volume of 0.85 ml of M-1 and transfer volume of 0.30 ml of M-2.

Initial conditions for the length of the (isocratic) elution steps and for the step-gradient profile ($\Delta\phi$ per step) to be applied in chromatogram simulation are based on a proper interpretation of the $\ln k$ versus ϕ plots of the analytes. This crucial information of the pesticides involved is given in Fig. 6.25. This figure indicates that a mobile phase containing about 30% methanol can be used for the first analyte (metamitron), providing there is an adequate clean-up (criterion I) and sufficient separation from bentazone. However, the length of this first step is limited because of the poor resolution between monuron and metribuzin (criterion II). For these analytes and DNOC, elution with a mobile phase containing about 50% methanol will be more appropriate. Finally, an eluent containing about 70% methanol will provide proper elution and separation of diuron, linuron, dinoseb and dinoterb (criterion III). Using this information, a two-step gradient elution meeting the separation criteria mentioned above was rapidly found by applying the rational optimization procedure (see Fig. 6.22). The simulated separation conditions that were found to be suitable are illustrated in Fig. 6.26 which also shows the good agreement between the simulation and experiment. However, during analyses at low levels, it appeared that diuron was not sufficiently separated from the distortion peak caused by the eluent-switch of the step-gradient. Making a concession to the total time of analysis (from 10 to

15 min), a suitable one-step gradient elution was rapidly found by chromatogram simulation, using mobile phases of 40 and 65% of methanol in 0.03 M phosphate buffer (pH 3.2).

To maintain the selected separation power, two 50×4.6 mm i.d. Microspher C-18 ($3 \mu\text{m}$) columns (instead of one 100×4.6 mm i.d. column) were used for the on-line clean-up of the extracts with column switching. The conditions for this, a clean-up using 1.6 ml of M-1 (40% methanol) on C-1, and a transfer volume comprising 5.9 ml of M-2 (65% methanol) and 10 ml of M-3 (65% methanol) were calculated using the computer program. The results are in good agreement with the experimental data, as illustrated in Fig. 6.27 which shows the RPLC analysis of a ground-water sample spiked with 1–2 ppb of each of the pesticides. By applying a variable wavelength detection program, all pesticides could be assayed in ground-water at the required detection limit of 0.1 ppb ($S/N = 3$).

Recoveries, made at levels between 0.2 and 2.8 ppb ($n = 10$ for each pesticide), ranged from 67 to 114% with relative standard deviations (RSDs) of 4.5–8.5%.

6.6. CONCLUSION AND TRENDS

The work presented above demonstrates that the combination of coupled-column RPLC and UV detection is a practical and flexible technique for the determination of po-

TABLE 6.8

RPLC-UV CHARACTERISTICS^a OF NINE POLAR PESTICIDES

Pesticide	C-18 retention (k')	λ_{max} (nm)	ϵ_{max} (l/mol cm)
Metamitron	2.4	308	10000
Bentazone	3.8	217	25000
		229	17000
Monuron	6.0	244	16600
Metribuzin	6.6	294	9262
DNOC	9.2	265	9500
		365	5200
Diuron	18	244	17000
Linuron	31	244	17400
Dinoseb	99	265	8100
		365	4300
Dinoterb	116	265	8100
		365	4300

^aData experimentally determined on a $3 \mu\text{m}$ Microspher C-18 column with a mobile phase of methanol/0.03 M phosphate buffer (pH 2.9) (40:60; v/v) and UV photodiode array detection.

lar pesticides. Basically, two different strategies are proposed: the development of single-residue methods (SRMs) for the rapid, sensitive and selective assay of a single polar pesticide and the development of multi-residue methods (MRMs) for the simultaneous determination of a group of polar pesticides with widely different polarities.

The SRM methodology is useful for screening polar pesticides in environmental aqueous samples, giving LODs at the required level of $0.1 \mu\text{g/l}$ for analytes with reasonable RPLC-UV properties such as phenylurea herbicides, benzimidazoles and triazinones.

Although this work has been focused on the determination of polar pesticides, the flexibility, sensitivity and selectivity of this approach seems also appropriate to the analysis of moderately polar and non-polar compounds.

The simultaneous determination of a group of polar pesticides including analytes with poor UV detectability and little retention usually requires the preparation of concentrated extracts prior to the LC analysis in order to obtain sufficient sensitivity. Therefore, the proposed MRM methodology is based on the processing of extracts by coupled-column RPLC for on-line clean-up and step-gradient elution for the efficient separation of ana-

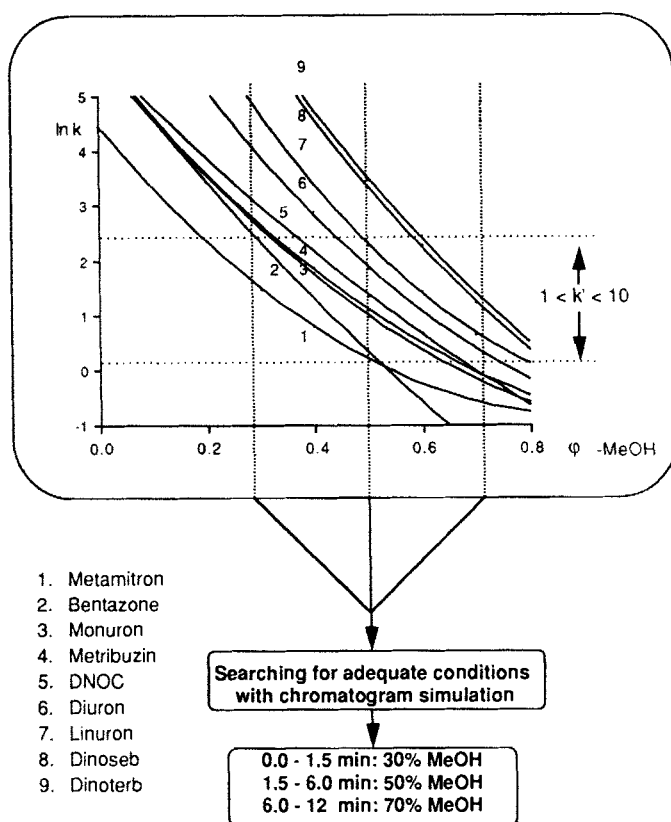


Fig. 6.25. Retention behaviour of nine polar pesticides on a $3\text{-}\mu\text{m}$ Microspher C-18 column using methanol/ 0.03 M phosphate buffer (pH 3.2) as the mobile phase.

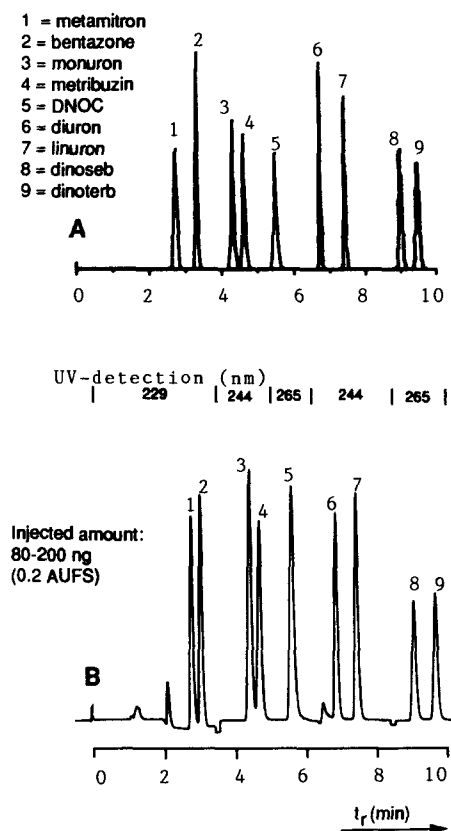


Fig. 6.26. (A) Simulated chromatogram of a two-step gradient elution with initial conditions (see Fig. 6.25) established with computer program. (B) Experimental verification of the simulated chromatogram on a 3- μ m C-18 column (100 \times 4.6 mm i.d.). Injection, 50 μ l of standard solution.

lytes. A powerful tool in method development is computer-aided chromatogram simulation, which can rapidly find proper conditions for clean-up and separation. However, suitable conditions must be found step-by-step, using chosen elution profiles which makes the optimization procedure rather dependent on the ability of the analytical chemist. Improved results, in the form of truly optimal conditions and reduced optimization times, may be expected from the application of an automated optimization procedure. Such an approach is currently being investigated for one-step gradient elution.

Because of its broad scope of application, long-term stability, ease of everyday use and low cost, UV detection is very attractive for PRA and thus in productive method development. However, at low wavelengths, UV detection is not very selective and, despite the demonstrated increase in selectivity using coupled-column RPLC, one might still expect difficulties in obtaining sufficient selectivity for some matrix/pesticide combinations. In order to exploit the attractions of UV detection, new trends in more selective column switching should be investigated in PRA.

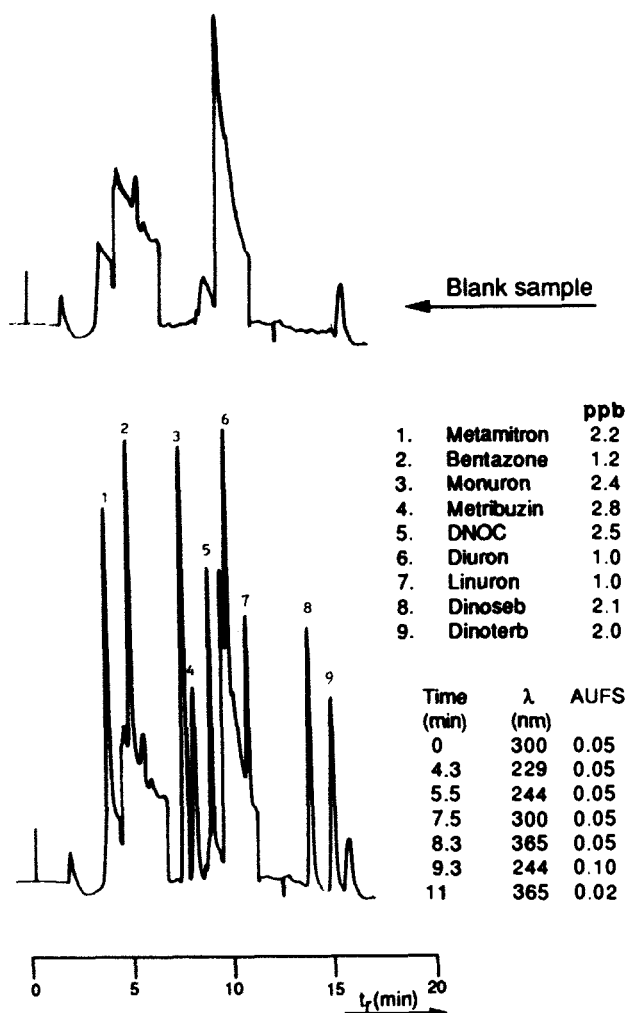


Fig. 6.27. RPLC-UV of a ground-water sample extract spiked with the pesticides at a level of about 1.5 ppb, and the corresponding run of the blank sample using column-switching and one-step gradient elution (see Fig. 6.20) for on-line clean-up and separation, respectively. C-1 and C-2, 50 \times 4.6 mm i.d. column packed with 3 μ m Microspher C-18; M-1 and M-2, methanol/phosphate buffer (pH 3.2) (40:60, v/v); M-3, methanol/phosphate buffer (pH 3.2) (65:35, v/v); flows set at 1 ml/min; 100- μ l injection on C-1 of a 500-fold concentrated sample extract; clean-up volume, 1.6 ml of M-1; transfer volume, 5.9 ml of M-2 and 10.0 ml of M-3.

One of these trends is the use of high-efficiency separation columns packed with internal-surface reversed-phase (ISRP) materials [99,100]. Such columns can exclude large molecules from the pores and separate small molecules (analytes) with good capacity and efficiency. They have been applied well to drug analysis with direct serum injection [101,102]. Recently, an ISRP column was successfully applied as a first column for the

coupled-column analysis of flucycloxuron residues in raw apple extracts [103]. The RPLC-UV analysis of acidic pesticides in natural waters is always hampered by the presence of a large and broad interference peak (hump) in the base-line, arising from humic substances [58,104,105]. It was shown that for the determination of chlorophenoxy acid herbicides in environmental samples [106] and mammalian tissues [107], the application of a first column packed with ISRP material provided more selectivity than conventional C-18 or C-8 material.

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Chapter 7

Liquid chromatographic determination of phenols and substituted derivatives in water samples

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7.1. INTRODUCTION

Phenol and substituted phenols such as chlorinated phenols and related aromatic compounds are known to be widespread as components of industrial waste. These compounds are made in the course of many industrial processes worldwide, for example in the manufacture of plastics, dyes, drugs and antioxidants and in the pulp and paper industry. Organophosphorus and chlorinated phenoxyacids also yield chlorinated and nitrophenols as major degradation products. 4-Nitrophenol was reported as a breakdown product after the hydrolysis and photolysis of parathion methyl in water and chlorinated phenols are formed by the hydrolysis and photolysis of chlorinated phenoxyacid herbicides [1–3].

Pentachlorophenol is the priority pollutant within the group of chlorophenols that has been most released into the environment. It has been used extensively worldwide as a pre-

servative for wood. Phenols are also breakdown products from natural organic compounds such as humic substances, lignins and tannins, that are widely distributed throughout the environment.

A hydrolysis step is involved in the pulp industry in order to extract the cellulose from wood. This uses large-scale processes whereby a liquid fraction, the lignocellulose, is formed as a by-product in the process containing high levels of phenolic components and their derivatives. These compounds also constitute an environmental problem owing to their possible introduction into rivers, lakes and/or seas. Chlorophenols from the bleaching process have traditionally attracted most of the interest in the analysis of industrial waste because of their high toxicity. This process has partly been altered in many Swedish pulp industries, whereby the chlorine has been changed for other less harmful chemicals.

Phenols and related compounds are highly toxic to humans and aquatic organisms, thus becoming a cause for serious concern in the environment when they enter the food chain as water pollutants. Even at very low levels, less than 1 ppb, phenols affect the taste and odour of water and fish [4].

New environmental regulations introduced throughout the world put greater pressure on industrial waste treatments. This fact has been realized by the pulp industries in both Europe and North America. Their waste waters as well as other industrial contaminated waste waters can in principle be treated by municipal sewage treatment plants. However, much effort and research are being invested in trying to make use of pulp industrial waste for ethanol production by fermenting these technical substrates. Total utilization of biomass is very important from environmental, industrial and agricultural points of view. It is an energy source that might be utilized to a much greater extent if proper technological solutions can be developed.

Method developments within these environmental areas are of great importance in helping to obtain the required selectivity and sensitivity, necessary for monitoring low levels of toxic substances as required by environmental regulations. New analytical techniques that can fulfil these requirements can be achieved by the use of chromatographic separation steps coupled with selective sample handling techniques. In this step, the sample is separated from the many matrix substances and simultaneously enriched. These goals are also reached by striving for more selective detection principles and techniques, which utilize the biological recognition offered for example by enzymes or antibodies [5,6], both in the sample-handling and the detection step.

A survey of column liquid chromatographic techniques for phenols and their substituted derivatives in water samples is given. The different parts of the systems are treated separately. Method developments for the determination of phenolics in pulp industrial waste waters and biotechnological samples from these sources are presented. Enzyme-based detection systems for phenolic compounds and their applications to waste waters are described.

7.2. COLUMN LIQUID CHROMATOGRAPHIC SEPARATION

The reversed phase separation mechanism provides by far the most commonly used CLC technique for the separation of phenolics. Silica materials of various kinds with C-18

as the bonded functional group give the best separation properties. Predictions of the retardation, separation and retention mechanisms involved in these separations are not easy to foresee *a priori*. Several groups have specialized in determining optimal separation conditions and separation parameters by using chemometrics and mathematical treatments of experimental results [7–9]. Although information is supplied by the manufacturers on the properties and stabilities of the stationary phases, in combination with various mobile phases, it is often difficult to relate this information to the specific separation problem that has to be solved. Trial and error experiments based upon the assumed probable interactions between analytes and the column support, and taking the chemical structure of the solute and the nature of the support into account, are therefore often a normal procedure.

The most commonly analysed priority pollutant phenols are chloro- and nitro-substituted phenols. However, a larger group of other substituted phenols are nowadays considered as toxic to nature [10–19]. Both isocratic and gradient elution modes are being used to separate these compounds. Early works by Horváth and Molnár showed the potential of gradient separations and achieved multicomponent separations of oxidized phenolics [20,21].

Marko-Varga and Barceló made a comparison study of the separation of 29 solutes having phenolic and related structures. The structures of these analytes were phenols with chloro-, nitro-, hydroxy-, methoxy-, ethoxy-, aldehyde- and carboxylic functionalities, and combinations thereof. Many of these compounds are normally found in waste waters from the pulp and paper industries [22]. The separations were made on polymer functionalized silica (Vydac), a polystyrene divinylbenzene polymer support (PLRP-S), a carbon (Hypercarb) and a silica C-18 (LiChrospher 100) stationary phase. The physico-chemical properties between these four column materials and the substituted phenols and related aromatic compounds and their impact on the retention characteristics were investigated. The difference in selectivity of the various columns for ten of these phenols is clearly seen in Fig. 7.1. The best separation was obtained by the use of the LiChrospher 100 column for these polar phenols and related compounds. The chromatogram in Fig. 7.1C also illustrates the poor separation efficiency of the carbon column although it should be added that the more apolar phenols showed a better resolution with this column. Both the PLRP-S and the polymer functionalized silica gave separation efficiencies between the two columns mentioned. These different stationary phases were further investigated with the 29 phenolics, which are all of major environmental concern [22]. The results from some of these phenols are shown in Fig. 7.2. These plots illustrate the linear relationship between the capacity factor (K') and the volume fractions of the organic modifier in the organic/aqueous mobile phase using the LiChrospher 100 column. The K' of the solutes often derives from the mixed retention mechanisms, and many of these are not well understood. The variation in capacity factor (K') versus the percentage of organic modifier used in the mobile phase can be used for the calculation of expected K' -values with certain mobile phase compositions [23]. The phenols were grouped into more and less strongly retarded analytes, illustrated in the two parts of Fig. 7.2. The amount of modifier was consequently adjusted for the two groups of phenols by using 80–20% and 20–5% levels, respectively of acetonitrile in the mobile phase. The characteristic breakpoint was found at 35% acetonitrile for the strongly retarded phenols while the same effect was found at 10% acetonitrile for phenols with less retardation. The separation efficiency of

this column was found to be highest at an eluent composition with a 20:80 mixture of acetonitrile and acetate buffer (50 mM, pH 4.2) for most of the solutes investigated. Typical separations for a number of phenols with this column are shown in Fig. 7.3. Breakthrough experiments and the resulting breakthrough volumes are also commonly used for calculating the expected K' -value from the relationship between this and the volume fractions of the organic modifier. Werkhoven-Goewie et al. used this approach to measure breakthrough volumes for phenols on reversed phase supports by using linear extrapolation of chromatographic retention data [24,25].

Coupled column techniques in CLC commonly used the introduction of a small precolumn prior to the analytical column. Large sample volumes can be eluted through these precolumns, with high flow rates, for simultaneous clean-up and trace enrichment. The coupled column systems offer reductions in the levels of matrix components and the enrichment of solutes for separation in the analytical column. The inclusion of the precolumn also results in higher sensitivity, controlled by the enrichment factors obtained. The same or similar types of supports are often used in the precolumns as for the analytical columns, i.e. carbon materials, octadecyl bonded silica phases and polymer supports of the polystyrene-divinylbenzene type [22,24,25]. After sorption of solutes on the precolumn, they are desorbed by a strong eluent, introduced into the analytical column and separated.

Coquart and Hennion [24] recently studied the retention times of a number of chlorinated and other substituted phenols on pyrolytic graphite carbon (Hypercarb) using a polymer support (PRP-1) and a C-18 silica support (LiChrosorb) for comparison. These

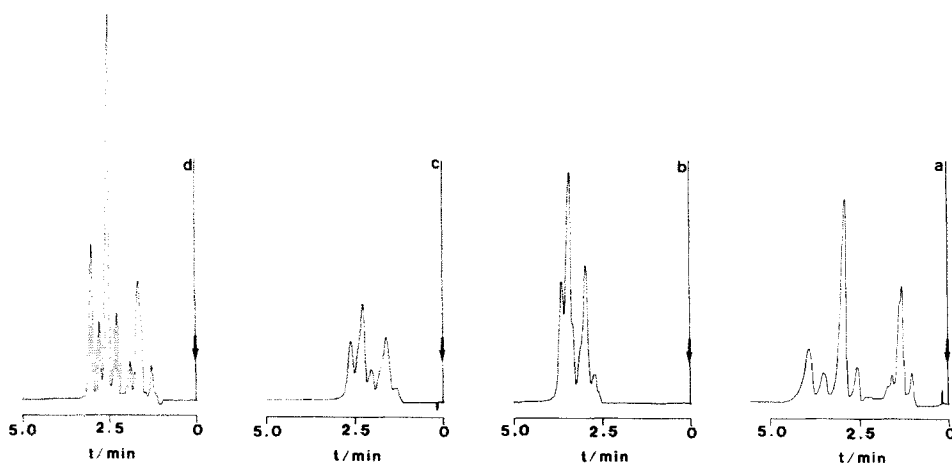


Fig. 7.1. Column liquid chromatographic separation of furfural, 3,4-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid, 4-nitrophenol, 4-hydroxybenzoic acid, 4-methoxybenzoic acid, 2-chlorophenol, 3-hydroxybenzaldehyde, coumarin and 3-methoxyphenol on (a) polymer functionalized silica (Vydac), (b) polymer (PLRP-S), (c) carbon (Hypercarb) and (d) silica (LiChrosphere 100) separation columns. Mobile phase; 50:50 (v/v) acetonitrile/acetate buffer (50 mM, pH 4.2) flow rate 1 ml/min, wavelength 280 nm, range 0.1 a.u.f.s; injection volume 20 μ l (reprinted with permission from ref. 22).

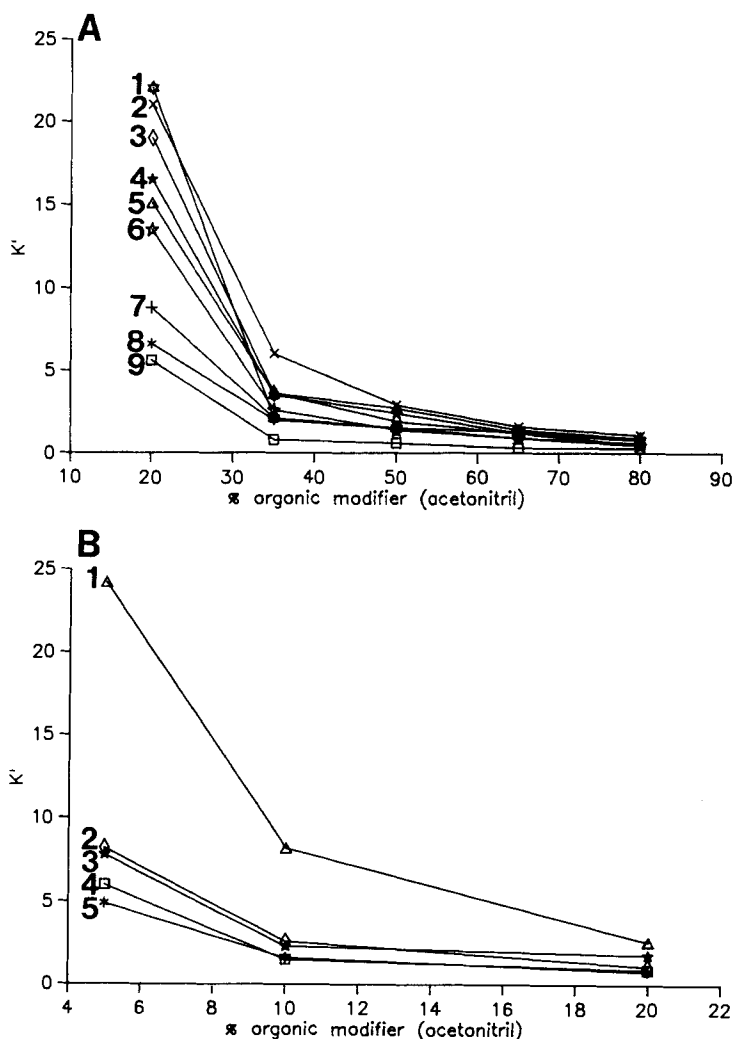


Fig. 7.2. Relationship between the capacity factor (K') and the amount of acetonitrile for more strongly retained phenols on a LiChrosphere 100 column: (A) (1) 2,6-dimethylphenol, (2) cinnamic aldehyde, (3) 2-chlorophenol, (4) 3-methoxybenzaldehyde, (5) *ortho*-cresol, (6) benzaldehyde, (7) 4-nitrophenol, (8) cinnamic aldehyde and (9) 3-methoxyphenol; for less strongly bound phenols. (B) (1) benzoic acid, (2) furfural, (3) 4-hydroxybenzoic acid, (4) 5-hydroxymethylfurfural and (5) 3,4-dihydroxybenzoic acid (reprinted with permission from ref 22).

investigations showed that the porous graphite carbon gave the highest retention times for pyrocatechol (1,2-dihydroxy benzene), resorcinol (1,3-dihydroxy benzene), phloroglucinol (1,3,5-trihydroxy benzene) and 2-chlorophenol. They led to the design of a precolumn for use for trace enrichment of 50-ml drinking-water samples spiked with these polar phenolics. Recovery values were found to be 52–100% using 20-ml samples. This

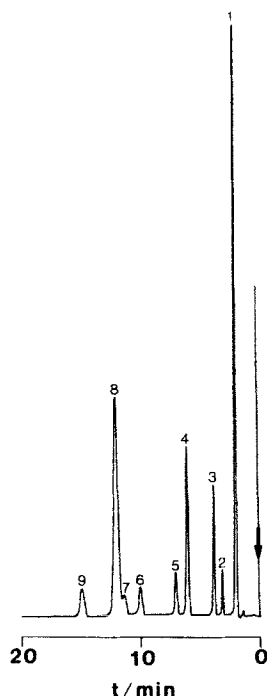


Fig. 7.3. Chromatographic separation on LiChrosphere 100 column, mobile phase acetonitrile/acetate buffer (50 mM, pH 4.2) (20:80), wavelength 280 nm, 0.1 a.u.f.s. (1) 3,4-dihydroxybenzoic acid, (2) furfural, (3) vanillin, (4) phenol, (5) 4-hydroxycoumarin, (6) 3,5-dimethoxyphenol, (7) 4-nitrophenol, (8) benzaldehyde and (9) *o*-cresol (reprinted with permission from ref 22).

indicates a breakthrough with a limited sample volume, which might be improved by using larger enrichment columns. Similar retention characteristics were found by Marko-Varga and Barceló for other polar phenolics using this carbon support [22].

Supercritical fluid chromatographic separation has also been used for several priority phenols [26,27]. CO₂ with the addition of chlorodifluoromethane (R-22) as an organic modifier was used as the mobile phase. The modifier chlorodifluoromethane has an increased solvation strength compared to carbon dioxide, which makes it suitable for separating polar phenolic compounds. Polar organic modifiers such as methanol, that are more commonly used, suffer from the drawback of having high critical temperatures and pressures [28].

7.3. SAMPLE HANDLING TECHNIQUES

The development of new sample handling techniques which can offer higher selectivity with larger capacity has been growing over the last 10 years. This fast development results from an increasing demand for the characterization and quantitation of progressively

lower amounts of compounds in complex biological matrices [29]. There is also a need for the development of automated systems for the screening of large numbers of samples. There is a trend towards increasing the selectivity in the sample clean up procedure and/or in the detection step in order to reduce the chromatographic requirements. This will result in the need for shorter analytical columns with simpler and faster separations.

The easiest and simplest sample handling uses a dilution in combination with a membrane- or ultra-filtration step. This reduces the level of interfering compounds and eliminates larger molecules, solids and colloids. The efficiency of removal of these matrix compounds is determined by the pore size distribution of the membranes used. Liquid/liquid extraction is still a very common sample handling technique although it requires large amounts of organic solvents, which is a disadvantage from an environmental point of view. However, the advantage of well-defined liquid bilayers with solute distribution between the organic solvent and an aqueous phase is still an efficient way of purifying samples.

The most widely used sample clean-up methods in the medical and environmental areas use solid-phase extraction techniques (SPE). The application and recognition of this technique have increased over the years because of the improvements in the technology [30–32]. The sample handling by solid/liquid extraction techniques can be based on precolumn technology, where one or several columns are connected on-line in a single or coupled column chromatographic system. The precolumn system is usually coupled via a column-switching arrangement to the analytical column, where multidimensional separations are often made. The clean-up can often be made for many samples (20–200), depending on the matrix complexity, before the columns are regenerated or changed. The solid phase extraction can also be made on disposable columns, where the column materials are of poorer quality. A wide range of different column materials exist today, which are available with a broad range of bonded silica phases, the most commonly used being C-8, C-18, diol, amino, amine, nitrile, alumina, and both strong anion- and cation-exchange supports [30–32].

These extraction cartridges are used for only a single clean-up, but can be used for several samples if their matrix levels are known and controlled. The stabilities of these phases are not normally in the same order as the analytical supports used in on-line SPE. Since most of the disposable SPE columns are made of silica materials, they are sensitive towards hydrolysis. This is of special importance, since most of the samples that are eluted through these columns are aqueous.

There are generally two modes for using solid-phase extraction for sample clean-up. The first is solute interactive SPE, involving a physicochemical interaction between the solutes and the stationary phase where the analyte is bound to the phase, as shown in Fig. 7.4A. The K' should be high for the solutes and kept low for matrix components. The solutes (named S in Fig. 7.4A) are desorbed in the next step by flushing the column with a strong eluent. Washing with a weak eluent can be used prior to desorption, to elute less strongly bound compounds. The second mode, matrix interactive SPE is used when the interaction between analytes and the stationary phase is poor while the K' values for interfering compounds are high as shown in Fig. 7.4B. This type of SPE is performed, for example, when the interferents to be eliminated have a very wide range of molecular weight distribution as in the analysis of anions in humic containing waters [33–35]. It is also

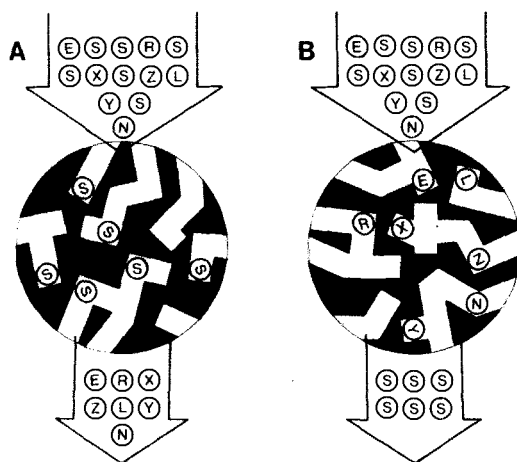


Fig. 7.4. Solid phase extraction principles. (A) Solute interactive SPE and (B) matrix interactive SPE. S, solutes; E, R, L, Z, X, N are matrix compounds.

applicable when the interferents represent classes of compounds ranging from polar to non-polar and the analytes of interest lack strong functional groups within their chemical structure which would enable strong bindings, as for carbohydrates (monosaccharides) where phenols are a major class of interferent [36–38]. The choice of operation is determined by the structure of the analyte(s) to be determined in the sample. However, the more common mode of the two is the solute interactive SPE.

The capacities of these SPE phases are generally determined by breakthrough measurements. These can be made by using a continuous flow through the column of a mobile phase containing the analyte(s). As more and more analyte is added to the column, a sample volume will be reached at which the analytes have bound to most of the available functional groups on the support and the analyte will begin to pass from the column. These breakthrough volumes can be used to determine adsorption isotherms and are important parameters to be found to give an understanding of the physico-chemical interactions taking place.

The columns are normally conditioned prior to use to eliminate residues from their production and to activate the column material. The most common procedure with a reversed phase disposable cartridge is:

- (1) activation of the stationary phase by an organic solvent, most often methanol;
- (2) conditioning, by equilibrating with water or more commonly a buffer of a certain strength and pH;
- (3) loading of sample;
- (4) one or several washing steps to desorb interferents;
- (5) desorption of the sample and its introduction into the analytical flow system.

The SPE columns may be used singly or in coupled column systems where several of them are connected in series. The choice will depend on the level and range of interfering constituents in the sample.

Clean-up by the use of SPEs has been used in both off-line and on-line modes. Both types of systems are open to automation. The advantage of on-line systems using SPE is that the risk of contamination is low while this is obvious in off-line systems.

High quality analytical CLC phases have been used in on-line systems while disposable supports with lower capacity and larger particle sizes have been used in off-line systems. The continuous use of the SPE column(s) in the on-line mode may result in memory effects, which is why a careful regeneration step is introduced to remove impurities from the column. This step is followed by an equilibration step before the next sample is injected. This is normally not a problem in off-line modes, since these columns are used for a certain number of analyses and then discarded and replaced by a new cartridge. The same principle is also used nowadays in commercial on-line sample clean-up systems, such as the Prospect which was developed by Nielen et al. in 1987 [39].

The use of supported liquid membranes, a new type of analytical sample-handling method, was developed by Audunsson [40,41]. The supported membrane is fitted into a two-part membrane separator and is pressed between two PTFE blocks with machined grooves. The liquid membrane is impregnated with a liquid phase and is positioned as a semi-permeable barrier between the two phases. The liquid chosen to impregnate the membrane needs to be immiscible between the two phases and is immobilized within the pores of the microporous film. The membrane therefore functions as a semi-permeable barrier between the donor and the acceptor phases [42]. Audunsson also developed mathematical equations to describe the transport of molecules through the liquid membrane [40,41]. Nilvé et al. used this method with simple permeation and with chemical reaction in the acceptor phase, for the selective enrichment of acidic herbicides [42–44]. The process is driven by a concentration gradient formed across the membrane. Continuous field sampling of chlorophenoxy acids (acidic herbicides) in natural waters was made with this unit using di-*n*-hexyl ether- and *n*-undecane-impregnated PTFE membranes [44].

This technique has also been used in the clean-up and enrichment of phenol and chlorophenols, since the latter are known breakdown products from acidic herbicides. Phenol, 4-chlorophenol, 2,5-dichlorophenol, 2,4,5-trichlorophenol, 2,3,5,6-tetrachlorophenol and pentachlorophenol were determined in investigations [45] using different pH in the two phases, thereby creating the gradient across the membrane. The chlorophenols were separated on a reversed-phase CLC system using electrochemical detection. The clean-up of phenols using supported liquid membranes was done off-line, but studies are currently being undertaken of on-line coupling to the CLC system [45]. Another type of membrane interface was used in an on-line configuration with CLC for the analysis of pentachlorophenol and other trace phenols in waste water [46]. Very recently, an additional on-line liquid-liquid extraction system was developed for a number of phenols [47].

Marko-Varga et al. [48] used microdialysis in a sampling unit for industrial waste-waters from the pulp industry. The stability and reproducibility of the microdialysis unit was investigated for 24 and 36 h continuous sampling in stirred shake flask experiments performed in our laboratories. The pH of the waste water was adjusted to 6.0 to obtain static conditions where the breakdown of organic matter can be almost eliminated. Carbohydrates were used as analytes in these experiments and recovery values above 90% were obtained. This means that no serious clogging effects or other interactions take place

on, or close to, the polycarbonate membrane surface, to interfere in the diffusion process. Applications to phenol analysis with similar waste waters are currently under study, and show high recovery values, similar to those for the carbohydrates.

7.4. DETECTION

7.4.1. Spectrophotometric detection

UV-spectroscopy and electrochemical detection are most commonly used for the analysis of phenols. Amperometric oxidation will, in general, yield higher sensitivity by about an order of magnitude or more, depending on the structure of the phenol. However, the combination of UV (especially with diode-array) and amperometric detection provides an increase in selectivity, which is specially important in complex biological and environmental samples.

In environmental analysis, UV-diode array detection is an alternative technique for the search for particular pollutants in the samples and to provide identification of the substances present [11,18,49]. This can be made by matching the spectra of peaks with library spectra for confirmation. Many refinements using DAD data are available today such as the reduction of noise mathematically, peak purity investigations and the ratios of absorption at different wavelengths [49].

7.4.2. Electrochemical detection

Galetti et al. [50] found a great advantage using EC-detection of common breakdown products from lignin such as vanillin (4-hydroxy benzene), syringaldehyde (3,5-dimethoxy-4-hydroxybenzoic acid) and *p*-coumaric acid (cis- β -phenylacrylic acid), in the presence of high levels of nitrobenzene. This was not feasible with the UV-detector which is affected by other non-phenolic UV-absorbing compounds. In a comparison of UV and EC detection principles, it was reported that despite a decline in the signal-response of the working electrode, EC was to be preferred in applications owing to its higher sensitivity and selectivity [51]. When method development is made with electrochemical detection [52], the choice of the operational potential will always depend on the oxidation and/or reduction potential of the solute(s). This can be determined via a volumetric analysis which may be made using a hydrodynamic voltammogram. This possibility is given by many commercially available CLC electrochemical detectors.

The chosen operational potential will in most cases be a compromise between the optimal faradaic current and the lowest level of background current for each solute.

Electrochemical oxidation of phenolics requires the use of high applied potentials, around 1 V versus SCE which opens up the possibility of fouling of the electrode. Phenolic analytes, especially at higher concentrations, have a tendency to polymerize upon oxidation [53,54]. The structure of the phenol, the electrode material and the experimental conditions will determine the effective oxidation potential of these compounds. Upon oxidation, phenol, and *o*- and *p*-dihydroxybenzenes form *o*- and *p*-quinones, respectively. Two metahydroxyl groups in the ring will increase the oxidation po-

tential some 500 mV. Chiavari et al. [55] found the oxidation potentials for commonly occurring phenols in industrial waste waters to be between 0.5 and 1.25 V, using the cyclic voltammetry technique and a glassy carbon working electrode. A voltammetric-amperometric dual-electrode electrochemical cell configuration has also been used [56]. The electrochemical cell is operated by scanning the potential of the upstream electrode and detecting the amperometric current at the downstream electrode of the cell. With this method, only reversible electrochemical redox couples will give an amperometric signal at the downstream electrode. The ratio of the current of the two electrodes will give the "collection efficiency" which is a value that can be used to characterize the analyte and its detectability in electrode operations at a mass-transport-limited plateau [56]. A similar approach can be made by using two amperometric flow cells coupled in line where in the first coulometric cell all or most of the phenols are oxidized ($E = 1.0$ V) and then reduced ($E = -0.2$ V) in the second cell [57]. Bromo-, cyano- and iodo-substituted phenols (used as pesticides) were monitored. The use of this voltammetric CLC flow cell and measurement of the anodic signals of the aromatic hydroxyl groups, made it possible to determine them in the picogram range [58].

Although much work is carried out to increase the selectivity, as in the methods described above, many phenols are not well suited for these electrochemical operations. This means that the potential applied, which is normally high in the amperometric detector units today, will open the possibility for other matrix components in the sample to be oxidized. This leads to higher background currents that will reduce the detection limits. Another problem encountered with the use of the high applied potential is the competition between oxidation of the phenol and the electro-polymerization which takes place at the electrode surface. The polymeric products are adsorbed on the electrode surface, thereby fouling the electrode, and are seen as a decline in signal response with time [4].

The normal procedure in laboratories is to use the electrode until it reaches a lower level of acceptance for a standard injection of a reference phenol(s). At this stage, the background current and the noise of the working electrode have increased considerably. During the continuous operation of such a CLC-EC system, it is of major importance to use internal standards [51]. The data need to be carefully calculated by appropriate methods in which the decline in signal with time is taken into consideration.

In order to recover the working electrode, its surface is cleaned by polishing with fine emery paper. The original properties are sometimes difficult to regain after such polishing treatment [58]. Gradual poisoning of the electrode surface can be corrected for in EC-detection by measuring the product of the peak height and the peak width at half-height [59].

The reference electrode should also be non-polarizable and constant in order to allow reproducible measurements. The reference electrode is often also used as the counter electrode in a two-electrode flow cell. This can be achieved if the faradaic currents are kept low. Normally, AgCl /NaCl or AgCl /KCl reference electrodes are used, where the electrode might be a solid Ag capillary with an electrolysed chloride layer outside. The constant chloride level is added by dissolving some tenths of mM of the chloride salt in the eluent. The other position for the reference electrode is outside the cell with Luggin capillary connection. The criterion for the reference electrode is that it should be kept non-polarizable throughout the chromatographic separation. If not, that is, if the potential

of the reference electrode is shifted, even slightly, the applied potential of the working electrode will not be constant.

The lifetime of the working electrode is very much dependent on the purity of the sample. In this context, it is worthwhile undertaking more extended investigations of appropriate sample clean-up steps in order to increase the electrode's lifetime.

Recent developments in electrochemical detection techniques have facilitated the sensitive and stable detection of phenolic compounds by the use of the pulsed amperometric technique (PAD) [4]. This uses a working potential sufficient to electrochemically oxidize the phenols, whereby a current is obtained, proportional to the concentration of the analyte. As the electrochemical conversion results in electrode fouling, a high oxidative potential pulse is applied next, resulting in the electrode being stripped of the blocking products formed. This pulse is followed by another pulse lower than the working potential, to reduce impurities on the surface and a new clean electrode surface is formed. The applied potential is thereafter stepped back to that originally applied for the electrochemical conversion of the solute(s). This way of solving stability problems in electrochemical detectors is not really new; the PAD technique was developed to its present form by Johnson and his group who used it for the analysis of carbohydrates, alcohols, amines and sulphur-containing pesticides [60]. Huesgen and Schuster [4] found that with this "self-cleaning" method, they could run 200 injections of severely contaminated river-water containing di-, tri-, tetra- and pentachlorophenols where the amount of all chlorophenols was about 4.72 ppm. A loss of 5% in signal response was obtained and a relative standard deviation with less than 2.5% for tetra- and pentachlorophenol in this investigation. PAD in combination with a preconcentration step made it possible to determine chloro-, nitro- and methyl phenols down to PPT levels [61]. By comparison with UV-detection (254 nm), a factor up to 100 in sensitivity was obtained.

The use of chemically modified electrodes (CME) circumvents some of the disadvantages of phenol detection using the electrochemical principles described above. As mentioned, the electrode material is of great significance to the electron flow from or to the electrode surface. A compression-moulded Kel-F-graphite composite electrode was used as the working electrode for phenol determinations [62]. The electrode was found to be compatible with many organic solvents with a linear operational range of six decades.

Another method of surface modification to the working electrode uses the deposition of copper onto a base-treated glassy carbon electrode. It was found to lower the operational potential needed for oxidation of phenols. The analytes might be polyhydroxic, such as carbohydrates or alditols, or common aromatic polyhydroxyl compounds like catechol, resorcinol and hydroquinone [63]. Some problems do arise from the simultaneous oxidative and reductive currents of these aromatics at certain chosen potentials (-0.1 to $+0.25$ V versus Ag/AgCl). This can naturally be overcome by the use of a higher operating potential, but then at the cost of an increase in background current. This CME was developed and used in CLC for the detection of phenols such as catechol, hydroquinone and 3,4-dihydroxybenzoic acid [64].

Another way of modifying the electrode surface is by the deposition of base-hydrolysed cellulosic film [65]. Access to the surface and penetration of the membrane is limited to molecules with small sizes. This also minimizes the risk of protein and/or macromolecular adsorption causing electrode fouling. The selectivity is very much improved in

complex samples but the improved signal response is also clearly seen by the injection of phenol reference standards.

7.4.3. Derivatization

Chemical derivatization and reaction detection in CLC and FI is a highly developed technique widely used to achieve the enhancement of both sensitivity and selectivity [29,66–70]. The reagent can be added in a reagent stream or immobilized on a solid support. In the field of catalytic reaction detection, most studies have been focused on immobilized enzymes in on-line flow configurations.

7.4.4. Enzyme based detection systems

Modification of the electrode surface can also be made by the incorporation of a biomolecule such as an enzyme. Enzymes, tissues and whole cells can be made compatible with electrochemical detectors [71–74]. The introduction of biospecificity of the amperometric detection can make it selective for the reaction in question.

Enzymes have been utilized for a long time as dissolved reagents in batch analysis. Nowadays, enzymes immobilized on solid supports or on electrode surfaces (enzyme electrodes) are used for detection purposes, and this is a focus of intensive work in food research and in the study of bioactive compounds in clinical, medical and biochemical areas.

Enzymes are proteins that speed up chemical reactions. Their inherent selectivity makes them very suitable as reagents for chemical analysis. As analytical reagents, enzymes possess some properties that have to be taken into consideration. They are flexible molecules, with a wide range of molecular weights, and complex conformations, which are generally linked to their catalytic activity. Oxidoreductases constitute the class of enzyme most often used in detection as biosensors or IMERs in CLC and FI systems. Pyridine-nucleotide dependent dehydrogenases and oxidases are the two types within the group of oxidoreductases which have found most applications in enzyme-based detection systems. The compounds most often produced and measured are NADH and H_2O_2 , detected by fluorescence, UV and electrochemical detection either directly or by a derivatization reaction. The cofactor in these enzymes may be soluble, and has to be added to the eluent in the flow system before or after the column, to obtain enzyme catalysis. Alternatively, the cofactor may be strongly bound within the enzyme structure, as with many of the oxidases. There are two different types of redox enzymes denoted dehydrogenases [75]. One is the group of enzymes which depend on a soluble cofactor NAD^+ or $NADP^+$ (nicotinamide adenine dinucleotide or its phosphate) and the other is the recently discovered group with a bound pyrroloquinoline quinone cofactor (PQQ). The $NAD(P)^+$ -dependent dehydrogenases constitute the largest group of redox enzymes known today, around 400 enzymes, whereas the group of oxidases contains about 150 different enzymes.

The first enzyme that was deliberately immobilized in 1916, was invertase. Nelson and Griffin [76] found that the activity of the immobilized enzyme was retained. Ever since, there has been an interest in immobilizing enzymes by attaching them to solid surfaces.

However, one of the first useful applications of immobilized enzyme preparation on a solid support only appeared in 1966 [77]. This used an immobilized enzyme reactor (IMER) containing urease in a flow stream, for the analysis of urea. The various flow configurations utilizing immobilized enzymes in IMERs and/or biosensors today, are illustrated in Fig. 7.5. Flow injection (FI) was introduced by Ruzicka and Hansen [78] and by Stewart et al. [79] in 1975–1976. Single IMER systems in FI now provide the most common way of utilizing enzymes as derivatization reagents. Single- or dual-component analysis is made in this configuration. However, some groups have specialized in multi-component analysis with the IMER-based FI technique. Multichannel IMER systems have been developed [80], whereby several analytes can be detected simultaneously, as shown in Fig. 7.5A. This development certainly approaches the achievements that can be obtained with CLC systems today. The limitation of the IMER-FI technique is still the need of new enzymes that can catalyse the reactions in a way compatible with many of the separations and determinations fulfilled by chromatographic separation techniques in laboratories today. When multicomponent analysis is required, especially of complex samples, a separation step is almost always needed, CLC is then the most commonly used separation technique. The positioning of the IMER in combination with CLC offers two possibilities: either, pre- or postcolumn as shown in Figs. 7.5B,C. In the precolumn mode, the substrate is converted into a product which is then separated. The postcolumn mode offers the possibility of using all available literature data since the substrates are first catalysed after the separation. Enzymes immobilized on electrodes and in conjunction with electrochemical transducers, shown in Fig. 7.5D, offer another mode of using enzyme-based detection systems in CLC. The combination of biosensors in CLC is still not very common, but offers great possibilities for the future [81,82].

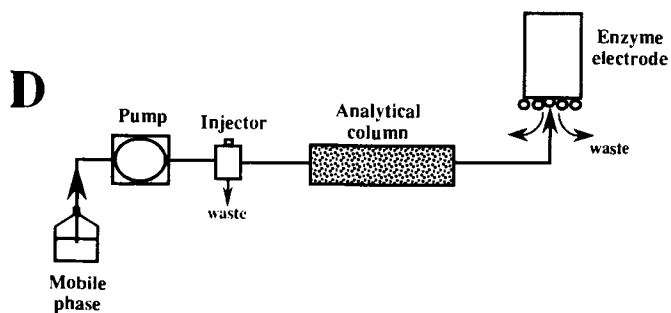
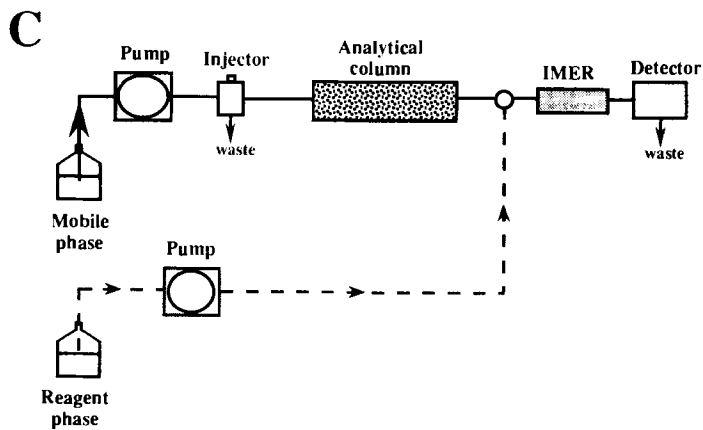
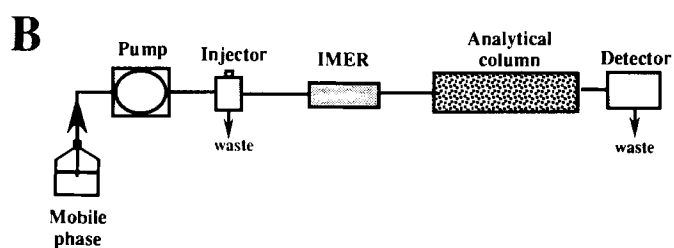
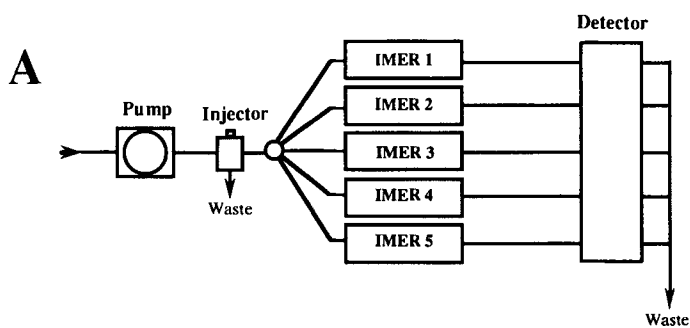
Clark and Lyons already foresaw in 1962 the construction of an enzyme electrode [83], and the first one was reported by Updike and Hicks who physically entrapped the enzyme using a dialysis membrane close to a Clark-type oxygen electrode [84].

Biosensors can be defined as comprising a bioactive substance, e.g. an enzyme, a microorganism, whole cells or an antibody, that can specifically recognise species of interest placed adjacent to a suitable transducing system as shown in Fig. 7.6. The most widely known is the enzyme electrode. So far, more than 1100 papers have appeared in the literature on glucose electrodes alone. An enzyme electrode can be used amperometrically, where the current between the working and the auxiliary electrode is measured, or by potentiometry where the potential difference generated between two electrodes (e.g. different types of ion selective electrodes) is measured. Below, only amperometric sensors are described and considered.

7.4.5. Fluorescence- and chemiluminescence detection

Derivatization techniques other than those using enzymes can be used to overcome drawbacks encountered with the commonly used detection principles such as chemical

Fig. 7.5. Flow injection and column liquid chromatographic flow configurations using immobilized enzymes. (A) Multi-IMER flow injection system, (B) precolumn CLC system, (C) postcolumn CLC system with a reagent flow and (D) postcolumn CLC system with enzyme electrode.



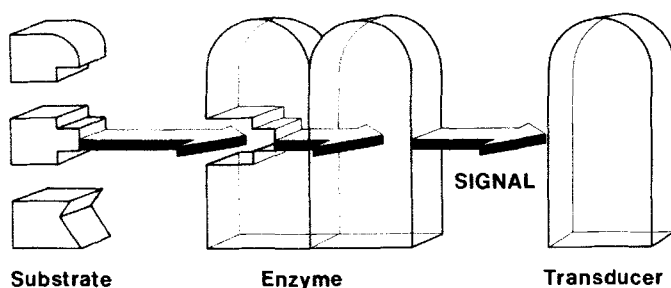


Fig. 7.6. Graphical illustration of the functioning parts of an amperometric enzyme electrode.

derivatization and reaction detection in CLC. Chemical derivatization can be made either in the pre- or the postcolumn mode. The choice will depend on the chemical structure and the physico-chemical properties under which the derivatization can take place. Chemical derivatization techniques in CLC are performed by addition of the reagent in an additional flow, after which the derivatization takes place. Catalytic reaction detection is another means of derivatization where the reagent can be immobilized onto a solid support. On-line systems for all derivatization reactions are preferred because they avoid the risk of contamination and eliminate tedious manual sample-handling steps.

De Ruiter et al. [85] developed a postcolumn derivatization procedure with enhanced fluorescence detection for substituted phenols. Photochemical decomposition was produced by photolysis of dansyl derivatives of the phenolic compounds. The UV irradiation leads to the formation of highly fluorescent dansyl derivatives which are several orders of magnitude more sensitive than non-irradiated derivatives [86]. The fluorescence gain factor with this reaction detection system was determined for a large number of chloro-, fluoro-, bromo-, nitro-, methoxy-, ethyl- and methyl phenols [86].

Chemiluminescence (CL) detection in CLC has increased considerably in the past decade owing to the low detection levels which can be achieved, in the range of 10–100-fold lower than for fluorescence detection [87,88]. The peroxyoxalate CL-system is the generally preferred detection system in both CLC and FI. Only recently has a more profound understanding of the reactions, i.e. the kinetic aspects, the selection of proper derivatization agents and the derivatization conditions, been sought [87].

Kwakman et al. developed a CLC method using both pre- and postcolumn derivatization for the determination of alkyl-, nitro- and chlorophenols [89]. They combined the two-phase dansylation, previously developed by De Ruiter et al. [85], with photolysis of substituted phenols and peroxyoxalate CL detection. Dansyl chloride was used, labelling the phenols by a two-phase dansylation. The second selective step is the detection by peroxyoxalate CL. The derivatization step is introduced by reacting the photochemically degraded dansyl chloride complex with 2-nitrophenyl oxalate and hydrogen peroxide.

Peroxyoxalate CL detection by reaction with enzymatically produced hydrogen peroxide utilizing immobilized enzyme reactors, has also been developed for several analytes [87]. These reactions have been used in combination with oxidases for the determination of choline, acetylcholine, formaldehyde, formic acid, carbohydrates, cholesterol, polyamines and L-amino acids.

7.5. APPLICATIONS

7.5.1. Background

Lignin and lignin breakdown products are present in both the cellulose and the hemicellulose part of hydrolysed wood. They may be very strongly bound within the cellulose structure and are then more difficult to exclude, or not so strongly bound thus making the exclusion process easier. The breakdown products from lignin yield mostly aromatic oligomers, dimers and monomers, and are removed from the cellulose in paper production in various ways, to eliminate the yellow colour of the paper. The levels of aromatics in the resulting waste, and the lignocellulose part, need to be controlled throughout the process, as well as before and after entering the sewage plant.

Wood hydrolysates will contain a broad spectrum of phenolic derivatives with for example hydroxy, methoxy, ethoxy, carboxy, and aldehyde substituents, and variations thereof. The complexity of the aromatic content will be determined by the harshness of the hydrolysis step. Hydrolysis by chemical treatment using sulphuric acid is considered to be a relatively harsh method, resulting in a broad diversity in the aromatic content. Hydrolysis methods such as enzymatic hydrolysis and mild chemical hydrolysis performed at lower temperatures using lower levels of acids, are considered to be "softer"

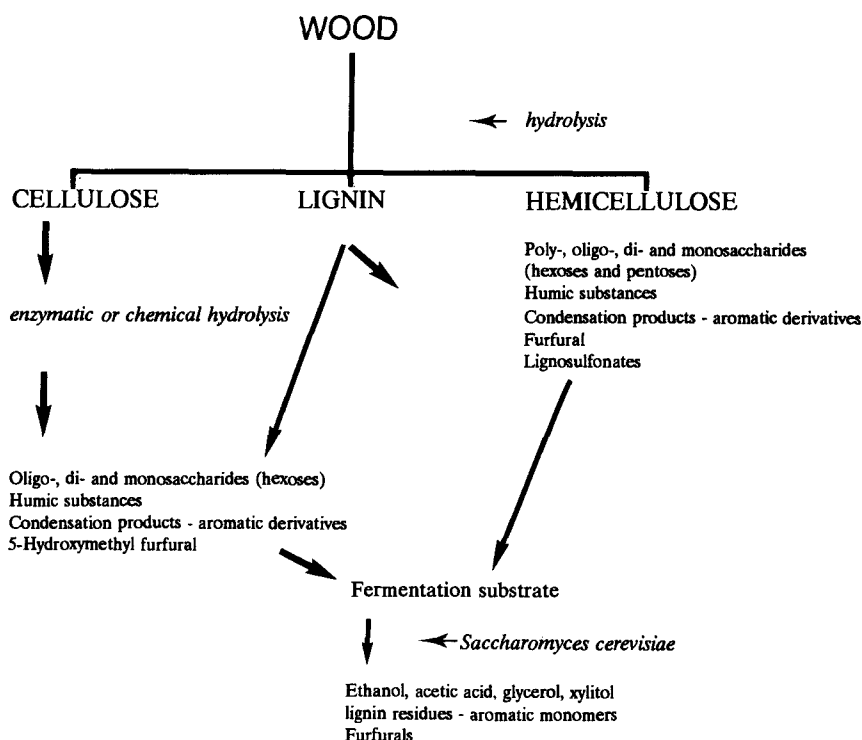


Fig. 7.7. Breakdown scheme of lignin in the pulping process.

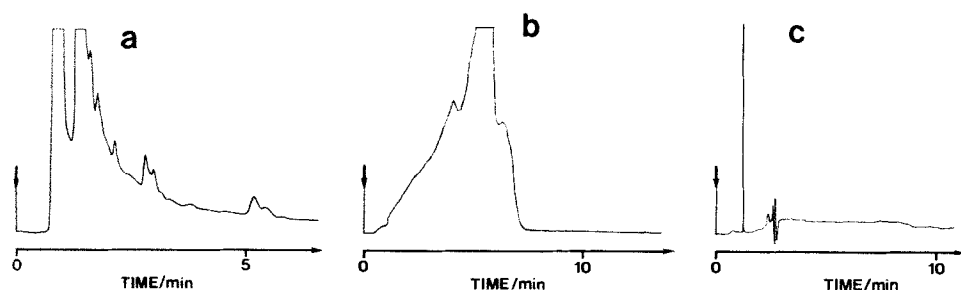


Fig. 7.8. Reversed-phase separations from Ebro river waste waters, sampled at the outlet from a papermill industry in south of Catalonia. (a) Sample directly from papermill effluent, (b) after desorption of the analytical column of the sample in (a), and (c) upstream river water (reprinted with permission from ref. 22).

methods. They also result in a breakdown scheme for the lignin of less complexity. The breakdown scheme of the lignocellulose hydrolysate is shown in Fig. 7.7. The composition of pulp industrial waste-waters also resembles these hydrolysates, where the nature of the waste will be dependent on the sampling point in the process.

Waste-water was taken from a paper-mill in south Cataluña, Spain, whose outlet is connected to the Ebro river extending then 20 km down to the Ebro delta and into the Mediterranean. This paper mill drained off its waste-water, without any purification, indirectly into the Ebro river. The second sample was taken 15 km further downstream in the Ebro river. The collected samples were filtered and injected and separated on a LiChrosphere separation column. The resulting two chromatograms are shown in Figs. 7.8a,c.

The sample from the paper-mill contains many separated peaks at the tail of the front. However, none of these could be identified using the retention times and UV-DAD spectra of 29 reference phenolic standards. The chromatogram shown in Fig. 7.8b was obtained after desorbing the column with an acetonitrile/acetate (85:15) eluent. This desorp-

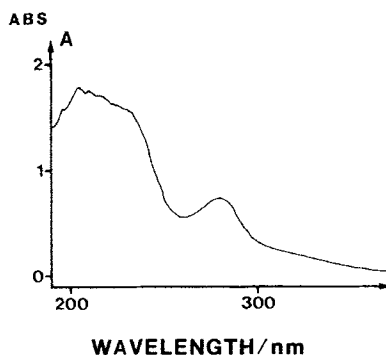


Fig. 7.9. Diode array UV-spectra taken after fractionation from chromatogram shown in Fig. 7.8b.

tion step was performed directly after the separation shown in Fig. 7.8a. Strongly bound compounds, probably non-polar lignin polymers, are eluted and the separation profile of these are shown in Fig. 7.8b. The UV-DAD of this fraction, shown in Fig. 7.9 was found to be similar for typical lignin compounds [90]. The sample downstream was found to contain only some minor peaks at the beginning of the chromatogram. As with the other sample, neither of these compounds could be matched with the model phenols. The paper mill sample was spiked with six phenolic compounds known to be breakdown products from lignin found in pulp waste-waters. This sample was separated using an optimized CLC method, as follows. The spiked sample was adjusted to below pH 1.5 to protonate the phenolics present in the sample. Next it was subjected to SPE, using a C-18 SEP-Pak cartridge, adsorbed on the clean-up column, and then desorbed by a strong acetonitrile/acetate mixture. After desorption using 2 ml of mobile phase, the samples were concentrated and then rediluted in the mobile phase. The purified sample, now less darkly coloured, was prepared for analysis by spiking with furfural, isovanillin (3-hydroxy-4-methoxybenzaldehyde), *m*-coumaric acid (cis- β -Phenylacrylic acid), 3-methoxyphenol, 3-ethoxy-4-hydroxybenzaldehyde and 4-nitrophenol after a seven-times dilution. The sample diluted in the mobile phase was then injected into the reversed-phase chromatographic system. A typical separation for this sample, with the clean-up described, is seen in Fig. 7.10 for six selected phenolics.

In this context, pollution and the risk of shortage of petrol-based energy sources have encouraged economists, politicians and scientists to consider alternative and renewable energy sources. One such energy source is the fermentation of cheap substrates. These substrates might be fast-growing "energy woods" such as willow (*Salix caprea*), but one can also use waste-water from the paper pulp industry and garbage tips as the carbon source. *Saccharomyces cerevisiae* (baker's yeast) is used to ferment the readily available sugars to ethanol (see Fig. 7.11) [91]. When industrial waste-water is used as the substrate, only the hemicellulose part of the hydrolysis product is utilized while the cellulose

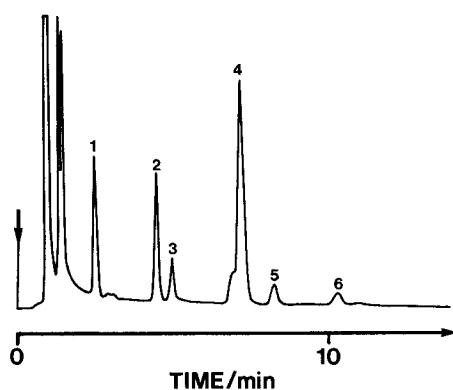


Fig. 7.10. A typical chromatogram of a spiked sample shown in Fig. 7.8a, after solid phase extraction, with the spiking of (1) furfural, (2) isovanillin, (3) *m*-coumaric acid, (4) 3-methoxyphenol, (5) 3-ethoxy-4-hydroxybenzaldehyde and (6) 4-nitrophenol, other conditions as in Fig. 7.8 (reprinted with permission from ref. 22).

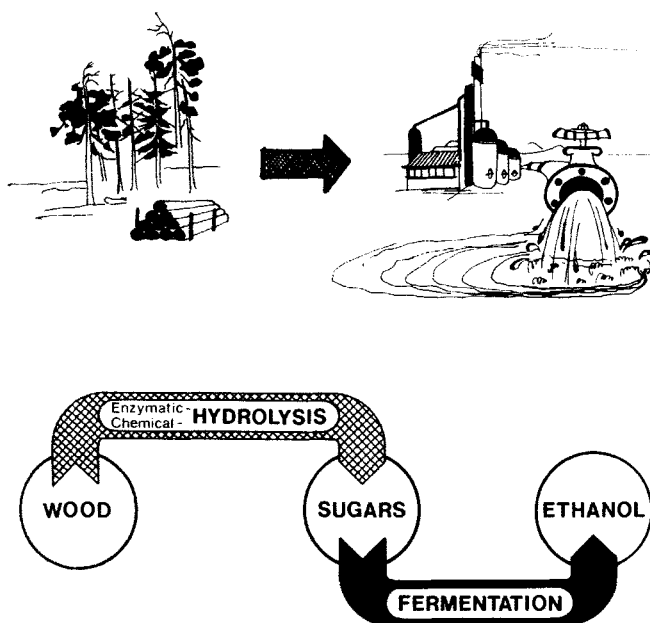


Fig. 7.11. Overview of the ethanol production using wood and waste water as the carbon source in the fermentation.

is used for paper manufacturing. By using fast-growing willow trees, both the cellulose and the hemicellulose can be mixed and used as carbon sources in the substrate.

It is noteworthy that lignocellulose materials have great potential for utilization, since they comprise about 95 % of the world biomass. Plant biomass, i.e. lignocellulose materials originating from agricultural residues, wood and industrial waste-waters is considered to be the most abundant renewable energy resource available for use as technical substrates to produce liquid fuels. Total utilization of biomass is very important from an environmental, industrial and agricultural point of view. It is an energy source that might be utilized to a much greater extent if proper technological solutions can be developed. High levels of poly-, oligo-, di-, and monosaccharides are present in these carbon sources for fermentation processes.

Wood hydrolysates comprise a very complex mixture of high, medium and low molecular mass compounds. Cellulose, hemicellulose and lignin are the three main constituents where the cellulose contains hydroglucose units of β -D-glucose units linked together by 1–4 glycosidic bonds, whereby the glucanic polymer chains are formed.

The hemicellulose can be described as a miscellaneous mixture of non-cellulosic, relatively low molecular weight polysaccharides, associated particularly, but not exclusively, with plant cell walls, mainly the secondary, but also the primary cell walls [92]. The classification of hemicellulose is based upon the chemical structure, as determined by the relatively few sugar residues found in the polysaccharides. The main ones are: D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic acid and 4-O-methyl-D-

glucuronic acid. The hemicellulose part will also comprise furan aldehydes, humic substances and condensation products, and easily dissolvable lignin and lignin breakdown products [93].

Lignin is a complex, highly branched, irregular, three-dimensional aromatic organic polymer. It consists of dehydrogenated polymers of phenylpropane subunits including coumaryl, sinapyl, syringyl, guaiacyl and *p*-coumaryl groups. These moieties are covalently linked together by a variety of bonds, mainly with β -aryl ether bonds of the lignin polymer monomers [93].

A collaborative project at the Chemical Center at the University of Lund in Sweden has for several years been occupied in finding new and more economical ways to use both technical substrates and solid wood as substrates for ethanol production.

There are many key factors to consider, but the main issues are as follows:

- (1) To find a hydrolysis process for solid wood that will result in a substrate composed of a more narrowly distributed series of phenolic derivatives. The hydrolysis is made by both chemical and enzymatic treatment. All the steps have to be made in a closed pilot-scale system with a limited number of operational steps. These steps need to be developed from both a chemical engineering and analytical point of view, since large volumes of liquids are circulated in the pilot plant, but the effects of using high pressures and temperatures in the hydrolysis steps need to be analytically evaluated.
- (2) The choice, production and reuse of the enzyme has to be taken into consideration.
- (3) To search for highly efficient microorganisms with the ability to withstand high inhibitory levels of aromatic breakdown products from lignin.
- (4) Mechanistic studies of pentose fermentations are needed for increased efficiency, since *Saccharomyces cerevisiae* only uses hexoses as substrates.
- (5) There should be selective and sensitive on-line monitoring of the processes described above.

There is a large research programme in Sweden for the biotechnological production and use of ethanol. This programme includes very early steps, such as a consideration of the carbohydrate content in soft- and hardwoods to be used as the raw material, up to the later steps, where the analysis of engine combustion is made. Different catalysts are investigated in the engines to help evaluate these results, and there is an ongoing study of more than 35 buses in Stockholm which use ethanol as fuel.

7.5.2. Fermentation as a means of utilizing industrial waste-waters

The industrial waste and other wood hydrolysates have very complex compositions, which even today are incompletely known. The wastes and hydrolysates that are used as substrates in ethanol fermentation processes are dark brown and highly viscous liquids [94]. Burtscher et al. [95] made reversed phase separations, using gradient elution programmes, for the analysis of phenols present in wood hydrolysates, as shown in Fig. 7.12. As can be seen in these separations, the complexity of the sample is very high.

The level of toxicity of the wastes used as fermentation substrates is an important factor for the overall yield of the ethanol process since they act as inhibitors of the microorganisms. It is therefore important to study the appearance and disappearance of the aromatics in the substrate and in the fermentation broth, in order to develop fermentation

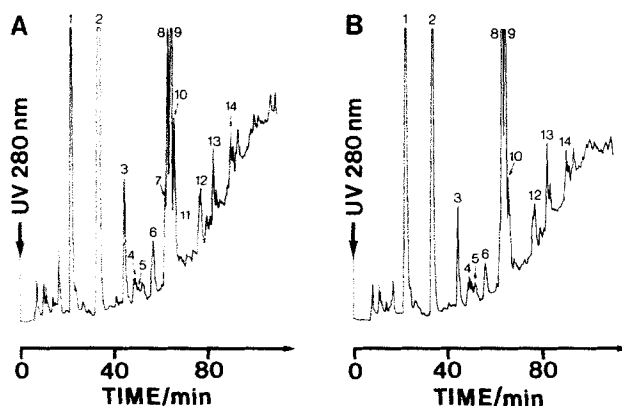


Fig. 7.12. Reversed phase gradient separations of hydrolysed poplar wood. (1) Hydroxymethyl furfural, (2) furfural, (3) *p*-hydroxybenzoic acid, (4) vanillic acid (4-hydroxy-3-methoxybenzoic acid), (5) homovanillic acid (3-hydroxy-4-hydroxybenzoic acid), (6) syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid), (7) vanillin (4-hydroxy-3-methoxy-benzaldehyde), (8) coniferyl alcohol (4-hydroxy-3-methoxy-3-phenylpropenol), (9) sinapyl alcohol (2-propen-1-ol, 3-(3,5-dimethoxy-4-hydroxyphenyl)), (10) syringe alcohol (3,5-dimethoxy-4-hydroxybenzoic acid), (11) acetovanillon (4-hydroxy-3-methoxy-actophenone), (12) 4-hydroxypropioiphenone, (13) propioguiaicon (2-methoxy-propioiphenol) and (14) pinoresinol (redrawn after permission from ref. 95).

conditions and microorganisms with the most appropriate capability to give the highest yield.

There is also a need, from an environmental point of view, to follow the levels of lignin oligomers and the phenolic content throughout the fermentation process. This allows one to follow the breakdown of these toxic phenolic compounds. In this way, new and better pathways may be found to reduce the toxicity of these wastes before releasing them into open waters whilst simultaneously providing an alternative-energy source.

7.6. CHARACTERIZATION OF LIGNIN AND ITS BREAKDOWN PRODUCTS

7.6.1. Gel permeation chromatography: diode array detection

As the phenolic oligomers and their breakdown products constitute such a large and heterogeneous group of compounds, the related analytical requirements are high. This fact makes it almost impossible to analyse high, medium and low molecular weight compounds present in the samples.

Gel permeation chromatography (GPC) in combination with diode array UV-spectroscopy is a powerful tool for the characterization of environmental samples [96] such as the breakdown products from lignin [96–98]. Industrial waste-waters and enzymatic hydrolysates of wood and their fermentation broths were subjected to analysis for the investigation of phenolics in lignocellulose materials. These would be, for example lignin oligomers, dimers and monomers, i.e. various phenolic derivatives. The characterization of

these waste-water samples was made with a sample-handling step using liquid/liquid extraction, in order to obtain a phase separation and distribution of the compounds present. Dichloromethane and water were used in the liquid/liquid extraction whereby three phases were obtained. A mixed phase appeared between the aqueous and organic phases. The size of this third mixed phase varied for the different waste and hydrolysate samples.

The aqueous and organic extracted phases were analysed by organic and aqueous GPC. The water phase from the liquid/liquid extraction was separated using a silica-based GPC column (Zorbax PSM 60 S) with water/methanol (90:10) as the mobile phase. The low amount of organic additive was used in order to eliminate hydrolysis of, and adsorption on, the silica support. This phase was found to contain major parts of the lignin polymers and their breakdown products [97]. The levels of lignin fractions in various molecular weight regions, and of aromatic compounds such as phenolics, aromatic aldehydes, acids, ketones and alcohols are high [99]. The separation of a 500-fold diluted sample of this phase is shown in Fig. 7.13. The upper chromatogram shows the collected waste-water while the lower chromatogram is of the same sample after 3 h of fermentation for ethanol production. As can be seen, the general trend is that the high molecular weight compounds represented by the peak at the beginning of the chromatogram decrease. This results in an increase in the smaller fractions (see peaks 2 and 3 in the second chromatogram). After 24 h fermentation, the separations look somewhat similar (chromatogram not shown) to that of the waste-water (chromatogram A1). However, the spectrum of this sample was different. It can be assumed that the increase in hydrophobicity of the fermentation broth resulting from the ethanol produced will increase the solvation of many of the aromatics with the more non-polar characteristics.

The organic phase of the liquid/liquid extraction was analysed by separation using a polymer-based GPC column (Bio-Beads SX-12, Bio-Rad) with dichloromethane as the

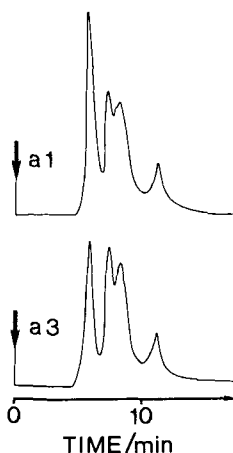


Fig. 7.13. Aqueous gel permeation separations of fermented waste-water samples after liquid/liquid extraction (aqueous fraction). Sample diluted 100-fold. Separation column, Zorbax PSM 60 S, mobile phase, phosphate buffer (25 mM, pH 7.0)/methanol (90:10, v/v), wavelength 280 nm; a1 and a3 are fermented samples (SSL) taken after 1 and 3 h, respectively

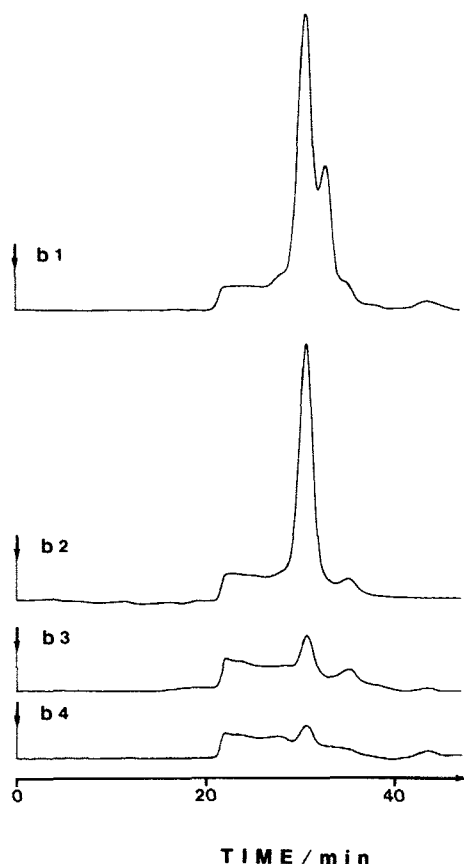


Fig. 7.14. Organic gel permeation separations of fermented waste water samples after liquid/liquid extraction (organic fraction). Sample (SSL), diluted 100-fold. Separation column (SX-12), mobile phase, dichloromethane (100%); wavelength 280 nm; b1, b2, b3 and b4 are fermented samples (SSL) taken after 1, 3, 5 and 24 h, respectively (reprinted with permission from ref. 97).

mobile phase. Figures 7.14 and 7.15 show chromatograms of one waste-water sample and the resulting broth after 0, 1, 3 and 24 h, and the separations of an enzymatically hydrolysed sample after 0, 1, 3 and 24 h. The latter sample is a potential substrate originating from a hydrolysate of a fast growing *Salix caprea*. Because of its potential as a carbon-source, the composition of this substrate and the fermented substrate is of great importance since it will become the industrial waste-water after fermentation. If such a substrate is chosen, the environmental effect of the whole process has to be taken into consideration.

As can be seen, in both of these samples there is a major breakdown of high molecular weight lignin oligomers with time, especially for the fermented *Salix caprea*. The corresponding UV-DAD spectra for the two samples were used for the identification and characterization of these samples. Scanning of each peak of the chromatograms provides

unique fingerprints for the specific compounds present in the sample. For more accurate confirmation, these can be fractionated and further analysed in a reversed phase CLC system.

7.6.2. CLC-mass spectrometry

Mass spectrometry has become an increasingly attractive technique as a result of the rapid developments and improvements in recent years. The analysis of polar or/and thermally labile phenolic compounds is difficult and troublesome by gas chromatography-mass spectrometry (GC-MS). These problems can be overcome by the use of CLC-MS with different interfaces, such as thermospray [100–104], particle beam [105] and fast atom bombardment [106]. Apffel and Perry [105] made a thorough investigation of the linearity by using particle beam-MS for the analysis of many phenols and related solutes. They paid special attention to non-linear behaviours at low concentrations and postulated

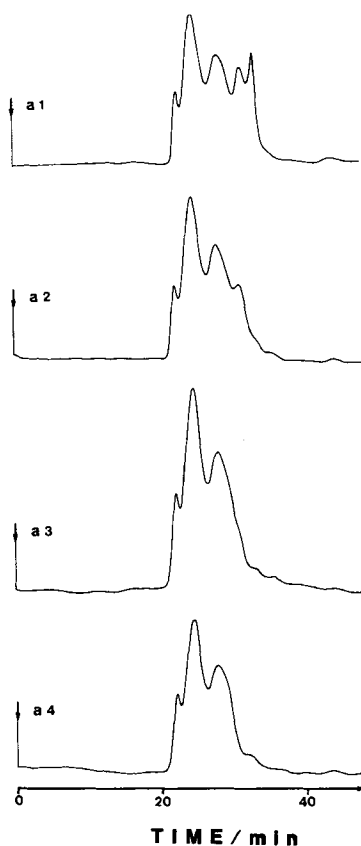


Fig. 7.15. Organic gel permeation separations of fermented waste-water samples after liquid/liquid extraction Sample (EH), a1, a2, a3 and a4 are fermented samples (SSL) taken after 1, 3, 5 and 24 h, respectively, diluted 100-fold, other parameters as in Fig. 7.14.

mathematical models for this non-linear region. TSP is the most popular among a number of interfaces for reversed phase CLC systems where organic/aqueous mixtures with acetonitrile or methanol as the organic modifier in the mobile phase are being used [103,107]. Ammonium acetate or ammonium formate are used as the volatile buffers in these mobile phases [100,103,107,108]. Relatively simple mass spectra are obtained using TSP as an interface for phenolics, carbamates, chlorotriazines, phenylureas, phenoxy acids, organophosphorus and quaternary ammonium compounds [100,103,107,108]. The main base peaks obtained in the positive-ion mode (PI) with the eluents described above are $[M + H]^+$ and $[M + NH_4]^+$. In the negative-ion mode (NI), the base peak obtained is much more dependent on the structure of the molecule. Anion attachment and proton abstraction are found to be predominant and similar to the results in conventional negative chemical ionization [103,109]. Compounds with high electronegativity, such as chlorophenols are found to give better response factors when cyclohexane is used as the mobile phase in normal phase chromatography [103]. The most common types of phenolics of environmental interest that have been analysed are the chlorophenols. Barceló characterized various chlorophenols using positive and negative ion modes in TSP-MS [100]. The PI mode showed no signal response for these chlorophenols (200 ng each). It is assumed that the ammonium ions are not able to protonate the chlorophenols. This was circumvented in the NI-mode, where the high intensity of $[M - H]^-$ results from the electron-attracting choline group in the aromatic moiety.

Only in two cases has the analysis of phenolic compounds and aromatics normally present in pulp waste-waters been investigated by CLC-MS techniques, using thermospray [108] and fast atom bombardment [106].

The nature of the reagent gas in thermospray interfaces will be determined by the composition of the mobile phase. Mobile phases containing formate or ammonium acetate as volatile ionic modifiers are commonly used in mixtures with an organic solvent. The choice of eluent was found to have a large impact on the optimization of both positive ionization (PI) and negative ionization (NI)-modes in TSP-MS [110,111]. The different clusters between the components of the mobile phase will determine the operative scan range. A compromise is always required between optimal chromatographic separation/resolution, and sensitivity regarding the choice of additives. Such an optimal eluent will also favour the choice of low operative scan ranges. In this case, coumarine and coumarine derivatives, hydroxy- and methoxy-substituted benzaldehydes and substituted phenols were analysed by Flow Injection TSP-MS in both the PI- and NI-modes. To avoid ions from the reagent gas, the scan range had to be operated from m/z values of 102 up to 250 in the PI mode, and from m/z 138 up to 400 in the NI mode. The relatively high range, especially in the NI mode, was due to the ions formed in the reagent gas. It was impossible to analyse low molecular weight compounds such as phenol, furfural and 5-hydroxymethylfurfural in both NI- and PI-modes and cresol isomers in the NI-mode. The eluent used was methanol/ammonium formate (50 mM, 50:50) with a flow rate of 1 ml/min.

Typical flow injection analysis peaks were obtained for a series of reference standards of phenolics using the PI mode of operation (see Fig. 7.16). 4-Hydroxycoumarin, 7-hydroxycoumarin and 3,5-dimethoxyphenol exhibited good responses. The base peaks corresponded generally to $[M + NH_4]^+$ for the coumarin derivatives, whereas the 3,5-di-

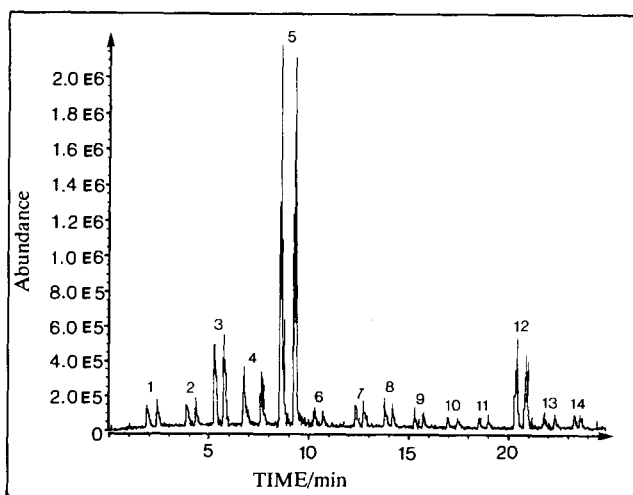


Fig. 7.16. Flow injection positive ion mode (PI) reconstructed TSP-MS spectra ion chromatograms of (ca. 1 μ g each) using 50:50 methanol/ammonium formate (0.05 M) as the carrier stream and a scan range from m/z 102 to 250. Compounds: (1) 3-ethoxy-4- hydroxybenzaldehyde, (2) 2,4-dihydroxy benzaldehyde, vanillin (4- hydroxy-3-methoxy-benzaldehyde), isovanillin (3-hydroxy-4-methoxy benzaldehyde), (3) coumarine (1,2-benzopyrone), (4) 4-hydroxy coumarin (4-hydroxy-1,2-benzopyrone), (5) 4-hydroxybenzoic acid, (6) *m*-coumarine, (7) isovanillic acid (3-hydroxy-4-methoxy benzoic acid), (8) vanillic acid (4-hydroxy-4-methoxy benzoic acid), (9) 3,4-dihydroxy benzoic acid, (10) 3,5-dihydroxy benzoic acid, (11) 4- hydroxy benzoic acid, (12) 3,5-dimethoxyphenol and (14) cinnamic acid (*cis*- β -phenylacrylic acid).

methoxyphenol had a $[M + H]^+$ peak. $[M + H]^+$ had an abundance between 10 and 30% for the coumarin structures. This pattern for the coumarin derivatives indicates that the proton affinities of these analytes are somewhat lower than ammonia and that they behave in a similar manner to for example, organophosphorus compounds in CLC-TSP-MS [101]. An implication of these results may be that these compounds exhibit an intermediate basicity, with proton affinity values equal to or smaller than that of ammonia, which favours the formation of both $[M + H]^+$ and $[M + NH_4]^+$.

Figure 7.17 illustrates the sensitivities for the various compounds in the NI mode of operation. As can be seen, they generally show a higher abundance compared to the PI mode. Comparison of the noise levels showed that the noise in the PI mode was higher because of the lower selectivity with this mode than the NI mode. All the coumarin derivatives have good response values (signal to noise ratio). The base peak usually corresponds to $[M + HCOO]^-$ which is to be expected when using ammonium formate as an ionizing additive in the mobile phase. The second most abundant ion usually observed in these analyses is $[M - H]^-$ except for 3-ethoxy-4-hydroxybenzaldehyde and 3-hydroxy-coumarin, where it is the base peak. In the former cases, the molecule can stabilize the negative charge much more easily, favouring the proton abstraction found to be typical of a negative ion chemical ionization mechanism.

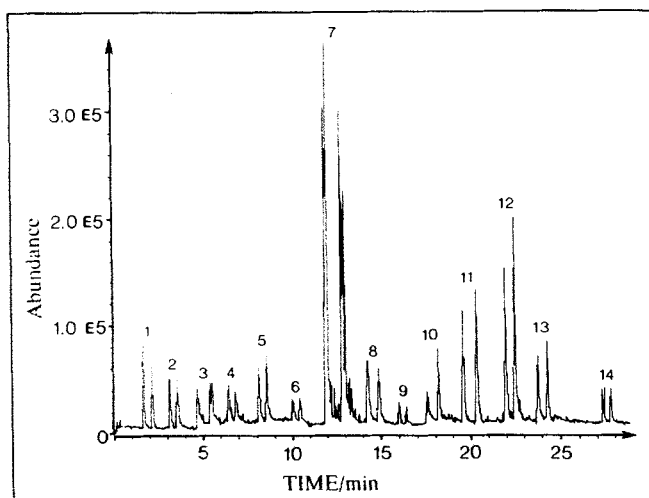


Fig. 7.17. Flow injection negative ion mode (NI) reconstructed TSP mass spectra ion chromatograms of ca. 1 μ g each using 50:50 methanol/ammonium formate (0.05 M) as the carrier stream and a scan range from m/z 138 to 400. Compounds: (1) vanillin (4-hydroxy-3-methoxy-benzaldehyde), (2) isovanillin (3-hydroxy-4-methoxy benzaldehyde), (3) 4-hydroxybenzoic acid, (4) 3,4-dihydroxybenzoic acid, (5) cinnamic acid (cis- β -phenylacrylic acid), (6) 3,5-dihydroxybenzoic acid (7) 7-hydroxy coumarin (7-hydroxy-1,2-benzopyrone), (8) 4-hydroxy coumarin (4-hydroxy-1,2-benzopyrone), (9) coumarin (1,2-benzopyrone), (10) *p*-coumaric acid (4-hydroxy-cis- β -phenylacrylic acid), (11) 3-ethoxy-4-hydroxybenzaldehyde, (12) 2,4-dihydroxybenzaldehyde, (13) 3,4-dihydroxybenzaldehyde and (14) 2,5-dimethoxyphenol.

After these studies, waste-waters from pulp industries were analysed using the following steps. Samples were obtained from the pulping process as a lignocellulose waste, after sulphite-pulping had been used as the hydrolysis treatment. Next, these samples were used directly with the addition of *Saccharomyces cerevisiae* (80 g/l) and neutralization to pH 6.0 with NaOH. Samples were then withdrawn from the fermentation, diluted 50-fold and filtered through a sterile membrane to remove solids and cells. Ligand-exchange separations were made, then the eluting compounds were fractionated and analysed by TSP-MS. The positive identification of *m*-coumaric acid is shown in Fig. 7.18. Here, the total ion chromatogram of the fermented waste-water sample fraction and the corresponding fractograms are shown. As expected, the response in the PI mode was negligible, but in the NI mode, ions were monitored at m/z values of 163 and 209 which correspond to $[M - H]^-$ and $[M + HCOO]^-$, respectively [112]. Other experimental conditions in the TSP CLC-MS were as described above.

A TSP mass spectrum of such a fractionated sample is shown in Fig. 7.19. The ion at m/z 255 in this spectrum corresponds to the adduct of $[M + (HCOOH)(HCOO)]^-$ which further confirms the identification. Several samples of fermented pulp waste-waters were analysed using CLC and TSP-MS as above and the resulting mass spectra. Although only *m*-coumaric acid has been identified in the first set of investigations by comparison with 25 phenolic and structured standards, it is clear from our mass spectral investigations that

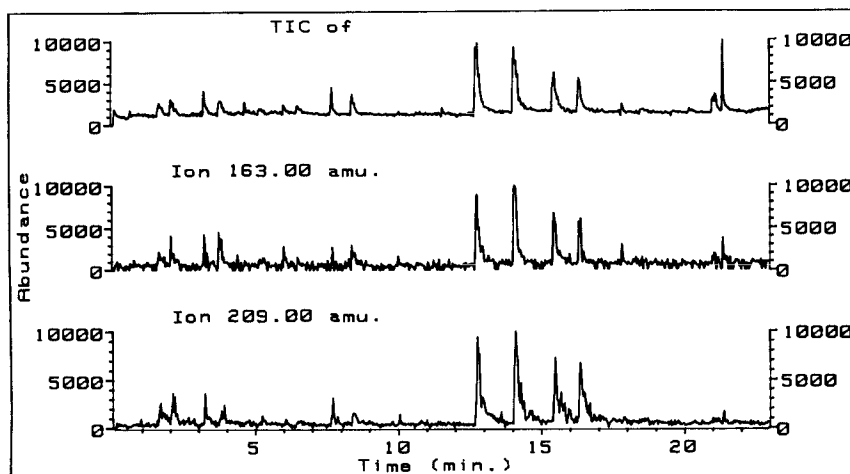


Fig. 7.18. Total ion chromatogram of fermented waste water sample and corresponding ion chromatograms, and some samples at m/z values of 163 and 209 in the negative ion mode.

other unidentified compounds are present, probably with phenolic structures and need to be identified.

Another aspect to consider is that these results imply errors in the quantitative analysis of carbohydrates in these types of processes. This is because there is co-elution of carbohydrates with other phenols or phenolic derivatives. This is very important since this lig-

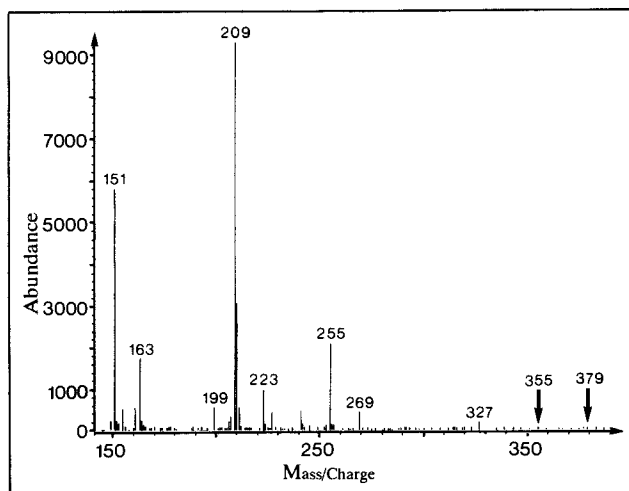


Fig. 7.19. Flow injection TSP mass spectra in the NI mode for *m*-coumaric acid. Main fragment ions corresponding to $[M - H]^-$, $[M + HCOO]^-$ and $[M + (HCOOH)(HCOO)]^-$ with m/z values of 163, 209 and 225, respectively.

and exchange CLC separation method is the commonly used standard methodology in the fields of for example biotechnology, biochemistry and microbiology [108].

7.6.3. Biosensors for phenols

The oxidations of different types of phenols and related structures are catalysed by different phenol-oxidizing enzymes, originating from a variety of sources. Generally two types of enzymes have been used for the development of biosensors. The first employs members of the copper-containing proteins often called "phenol oxidases". These are obtained from fungi, banana, potatoes and other sources. The second is a well-defined flavoprotein, phenol-monooxygenase (EC 1.14.13.7) [113]. The group of phenol oxidases is listed under several EC numbers. Catechol oxidase (EC 1.10.3.1) catalyses substrates with substituted catechol- and monophenolic structures. Synonyms such as diphenol oxidase, *o*-diphenolase and tyrosinase are often seen in the literature. Laccases (EC 1.10.3.2) are a group of enzymes that will act on both *o*- and *p*-quinones and also on aminophenols and phenylenediamine. Synonyms for this enzyme in the literature are polyphenol oxidase, phenolase and urishiol oxidase. Monophenol monooxidase (EC 1.14.18.1) will catalyse monophenolic and 1,2-benzenediol structures: its synonyms are tyrosinase, phenolase and cresolase.

Lignin peroxidase and aryl alcohol oxidase (EC 1.1.3.7) are two additional types of phenol oxidases, the first using hydrogen peroxidase as substrate and the latter is a hydrogen peroxide-producing enzyme, which are both responsible for lignin degradation [114,115]. Only the lignin peroxidase has been used for analytical purposes in flow systems [116]. However, these groups of enzymes have good potential as candidates for enzyme-based detection systems, especially since much work is under way to clone them and to introduce new and important functionalities into them [117].

More or less pure enzyme preparations are being used in biosensors as well as tissues and cells. It is often claimed that sensors based on tissues or cells are more stable and not so sensitive to harsh environmental surroundings [118] while this is commonly recognised for commercial enzyme preparations. This is because enzymes in tissues or cells are in their natural environments, making them more resistant, although their selectivity may not be as high as a result of other enzymes being present. Thus, most biosensors have been developed using enzyme preparations.

Depending on the source and origin of the enzyme, suitable purification procedures are commonly used to exclude impurities, inhibitors and other proteins present. These purification efforts are often worthwhile in crude preparations and even in pure preparations purchased from biochemical companies [73,119]. A large increase in enzyme activity was obtained in this way for such a preparation [73,119]. In general, highly purified and stable enzymes are always desirable for analytical purposes. The presence of other enzymes may interfere in the analysis of complex samples where the number of compounds that may react catalytically and consequently give a response, is unpredictable. Apart from an increased selectivity, one can envisage another advantage in the use of purified enzymes. In such preparations, there will be no competition for binding sites on IMER supports and electrode surfaces between the working enzyme and other proteins present in the crude preparation. This means that the IMERs can be made smaller for a given conversion effi-

ciency. Alternatively, they will allow an increase in the catalytic activity on the electrode surface, resulting in higher conversions from the higher enzyme density. This is especially important in CLC systems where any factor introducing band-broadening will damage the efficiency of the separation step. All these considerations could a priori drive the analyst towards a tedious and time-consuming purification step in order to increase the chromatographic plate numbers of the separation. Before any decision is made one also has to consider the stability of the enzyme. It is well known among biochemists that purity and stability of an enzyme extract seldom go hand in hand. In this sense, an increased selectivity and catalytic activity may be ruled out by the lack of stability. After considering all the enzyme types, with their substrate specificity, one must bear in mind that the nature of selectivity of the enzyme may also vary strongly depending on its source.

Method developments utilizing enzymes for phenol analysis have been made by immobilization on solid supports [120–122] contained in small columns (IMERs), and in or on electrode surfaces [113,123–126] for the construction of amperometric biosensors. The immobilization supports most commonly used are CPG (controlled porous glass) or polymer supports while the electrode material of broadest application is carbon paste. By choosing the IMER configuration, the surface area available for enzyme immobilization is very large and for the most commonly used CPG, silica and polymeric phases, it is around 20–300 m²/g. Depending on the size of the IMER, around 100 mg of support is commonly used. This is a very large surface area, if one considers that around 10% (w/w) of protein can be bound on the surface for optimal enzyme catalysis. This should be compared to the small electrode area of the surface, usually much less than 1 cm². This means in practice that 100% substrate conversion can be obtained by the use of an IMER. This is in contrast with the electrode configuration where product formation occurs by catalysis to levels usually below 25%, and in many cases with only about 2% of substrate conversion. IMERs can be optimized so that they are run above the catalytic capacity, meaning that the signal response is not influenced by their deactivation with time; 100% conversion can still be obtained. This is important from a stability and a calibrational point of view. Further, the use of IMERs does not suffer from signal variations with pH, flow rate, or other parameters of the flow systems. These variations are not likely to affect the enzymatic derivatization performance in the IMER. However, all these factors must be taken into consideration when the enzyme is immobilized in or on an electrode surface. Ortega et al. [121] made a comparison using immobilized tyrosinase for derivatization reaction detection with both IMERs and an enzyme electrode in FI and CLC (see below).

Fawer et al. [116] used both epoxy-activated polymer beads and a glass support with glutaraldehyde as a covalent coupling reagent for the immobilization of lignin peroxidase from *Phanerochatete chrysosporium*. Phenol, 3,4-dimethoxyphenol, 4-chlorophenol, 4-methylcatechol, 1,4-dimethoxybenzene and vanillyl alcohol could be used as substrates for this enzyme in a flow system. Zachariah and Mottola [120] used a tyrosinase IMER in combination with a carbon paste electrode incorporating immobilized hexacyanoferrate-(II)/hexacyanoferrate(III) as the redox mediator. The inclusion of poly(4-vinylpyridine) in the paste was advantageous in increasing the reversibility of the Fe^{II}/Fe^{III} redox couple. An increased signal was obtained by increasing the amount of modifiers, however, the background current also increased, so a compromise with 2% of modifiers was chosen.

Cliffe et al. [127] used another type of IMER, the membrane reactor. In this, mem-

branes used as the solid support are cut into discs and mounted between two polyethylene frits and sealed in a chamber. The membrane was an amino-derivatized membrane previously activated with glutaraldehyde, but it was found by the authors that a second glutaraldehyde step increased the coupling yield and increased the response. This might be because the enzymes are not bound only by covalent coupling but also by a network formed by the aldehyde to give a multilayer, immobilized on the membrane surface. The catalytic mechanism and the stability of lignin peroxidase were investigated [114,122] as well as the stability towards the use of the organic solvents dioxane, dimethylformamide and acetonitrile as the eluent [122].

Ortega et al. [121] used controlled porous glass (CPG) as the solid support material for binding tyrosinase from mushrooms. Wang and Naser [128] used a polyethylene cartridge, filled with the tissue from potato, in a flow system for the elimination of acetaminophen present as an interferent in samples. Polyphenol oxidase is the enzyme present in the tissue responsible catalysing the reaction.

Because an electron-transfer reaction is involved in the natural cycle of phenol oxidizing enzymes, enzymatic redox reactions are particularly closely associated with electrochemical transducers. There is considerable interest in the construction of "enzyme electrodes". More than 1200 papers have described the construction of various enzyme electrodes for glucose alone, and the greatest interest is clearly in the construction of amperometric enzyme electrodes.

The most commonly used electrode material so far has been carbon paste. This is made out of carbon powder mixed with a silicone- or paraffin-oil which contains the immobilized enzyme(s). Surprisingly, the biocatalytic activity is retained in this non-aqueous organic environment. The use of organic solvents and the capability of enzyme catalysis have been recognised for more than 25 years [129]. The potential for research which utilizes enzymes has generated intense activity in this field [130,131]. Klibanov's group has been one of the leaders in the development of enzyme catalytic reactions taking place in organic solvents. This and other work in this area has led to the development of organic-phase biosensors. These sensors have also been found to operate very well in organic solvents [132,133] and to operate best in the more apolar ones. "Superactivity" is an expression used to describe the improvements made by running catalytic reactions with enzymes in organic solvents in reversed phase micellar systems. In these systems, the enzyme is entrapped in a water cavity which is surrounded by a shell of surfactant molecules, and separates the enzyme from the surrounding organic solvent.

This is especially important for compounds having hydrophobic structures and which are poorly soluble in aqueous solutions. The use of biosensors in flow systems with organic solvents has also been shown [132–135].

Amperometric biosensors have been developed for the environmental monitoring of phenols. Only in some cases have potentiometric biosensors been developed, such as the clay-modified carbon-paste electrode for the determination of, e.g. phenol, aniline and nitrobenzene [136].

Neujahr and Kjellén [125] were among the first to develop enzyme electrodes for environmental applications and the determination of phenolic compounds. A cell paste of *Trichosporon cutaneum* was physically entrapped behind a dialysis membrane on top of a Clark-type oxygen electrode. An *ortho*-hydroxylating flavin monooxygenase present in

the yeast *Trichosporon cutaneum* is responsible for the catalysis of the reaction of phenols leading to the formation of an *ortho*-diol. In the second step, the diol is cleaved by a deoxygenase resulting in the total consumption of 2 mol O₂/mol phenols [137]. The decrease in oxygen tension is in good stoichiometric agreement with the concentration of analyte. This group continued with the same type of electrode using the enzyme phenol hydroxylase instead of whole cells [123]. Enzyme immobilization techniques, using the covalent coupling to modified nylon nets or AH-Sepharose 4B, adsorption on DEAE-Sephadex A50 or physical entrapment in polyacrylamide gel and the impact on sensitivity, were investigated. This pioneering work has been followed by sensors where the amperometric signal of product formation is measured rather than the decrease in co-substrate consumption as with the O₂.

It is always more difficult to make accurate measurements by following the reduction in a signal rather than its increase. This is especially true if one is measuring a physical property, such as the oxygen tension in a liquid, since the solubility of O₂ is also determined by parameters such as the salinity and temperature.

Tissue-based bioamperometric sensors have been developed using tissues incorporated into carbon paste electrodes [118,138]. These tissues contain polyphenol oxidases, which are copper proteins which catalyse the oxidation of certain phenolic compounds to quinones. It is interesting to note that each individual enzyme is rather substrate specific. It was found by Navaratne et al. [139] that eggplant polyphenol oxidase uses catechol as a substrate but phenol 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylglycol were not catalysed. Wang et al. [138] used a laccase from *Coriolus hirsutus* for the analysis of catechol, 4-methylcatechol and hydroquinone in a flow system. Non-aqueous eluents were used, such as methanol, ethanol, 2-propanol, 1-butanol and acetonitrile. The enzyme was immobilized onto a glassy carbon surface by mixing the enzyme solution with Eastman AQ polymer and then depositing it on the surface of the electrode. The Eastman AQ polymer is insoluble in these organic solvents and keeps the enzyme well protected in the polymer layer. Good stability was found for this sensor during 2 weeks.

Wang's group also used tissue from mushrooms and incorporated it into carbon paste electrodes (5 % w/w) [118]. This type of mushroom electrode had been reported earlier, but in a dual-electrode configuration for the analysis of catechol in a FI-system [138]. The mushroom carbon paste electrode was mounted in a thin-layer amperometric flow cell for the determination of monophenolic compounds such as phenol, the cresol and p-chlorophenol. The detection unit was used in the postcolumn mode in a CLC system. A reversed-phase silica C18 separation column was used with phosphate buffer/acetonitrile (70/30) as the mobile phase and an applied potential of +900 versus Ag/AgCl. One key factor to consider when coupling IMER or enzyme based electrodes as detection units in CLC is the additional band broadening obtained after the chromatographic separation. This happens because the plate number (N) of these systems are very much influenced by the postcolumn dispersion. A badly designed detection system might completely ruin an optimized chromatographic CLC separation resulting in severe loss of resolution. The additional broadening of bands arises from tubing, mixing tees, the construction of the IMER/biosensor, the flow, the adsorption characteristics on the enzyme support in the case of IMERs and on electrode materials and immobilization procedures (for biosensors). In both IMERs and biosensors the additional band broadening are in some cases

dependent on unfavourable enzyme kinetics [140]. The enzyme kinetics have to be optimized separately for each enzymatic reaction, to give total compatibility with the chromatographic separation.

Ortega et al. [121] made a comparison of enzyme-based detection systems where tyrosinase from mushroom was used as the biocatalytic component. Tyrosinase was immobilized on both a CPG support and on carbon electrode surfaces. A spectrophotometer and an electrochemical detector, respectively, were used as the transducers. The oxidation of phenolic analytes with molecular oxygen yields catechols, then subsequent dehydrogenation to *ortho*-quinones. These quinones are highly unstable in aqueous phases and readily polymerize to form coloured polyaromatic compounds which absorb in the visible region and can be monitored at 475 nm. The enzymatically-formed quinones and their derivatives can also be electrochemically reduced at low applied potentials. Ortega et al. made their comparisons between TYR immobilized in IMERs and on electrodes, using electrochemical and spectroscopic transduction in various FI and CLC flow systems (see Fig. 7.20) [121].

An IMER was used in the first case with amperometric detection and photometric detection (475 nm). In the second case, the detection system used for comparison, was an enzyme electrode, used as such, and then further coupled as a detection unit in CLC (see Fig. 7.20). The compounds analysed, e.g. dopamine and paracetamol, were important analytes in the biomedical area.

However, these results were also found to be useful for similar substrates such as the phenolic compounds of environmental interest, phenol, catechol and *p*-cresol. In fact, several phenolics active towards this enzyme are interesting both medically, because it is

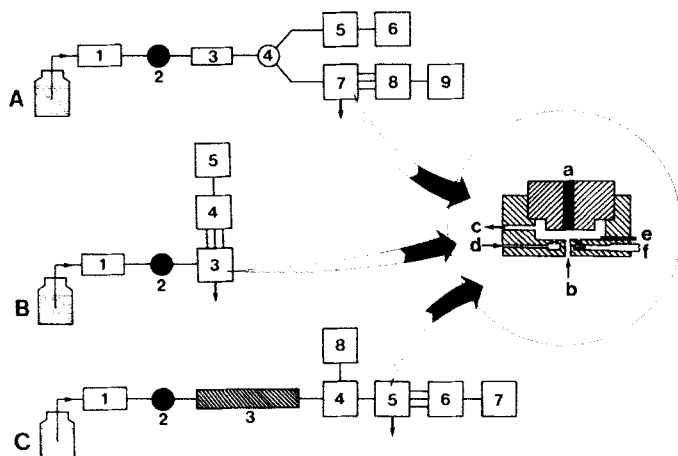


Fig. 7.20. Flow injection (A) and (B) and column liquid chromatographic (C) flow manifolds. (1) Pump, (2) injection valve. (A) FI system with either photometric or electrochemical detection using an IMER, (3) IMER, (4) switch valve, (5) photometric flow cell, (6) recorder, (7) electrochemical flow cell, (8) potentiostat and (9) recorder. (B) FI- system with enzyme electrode, (3) electrochemical flow cell, (4) potentiostat and (5) recorder. (C) CLC system with (3) analytical column, (4) potentiostat, (5) recorder, (6) potentiostat and (7) recorder.

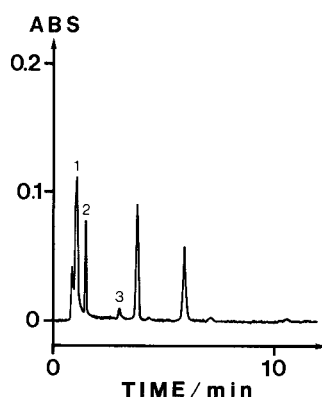


Fig. 7.21. Reversed-phase separation of a sample from pulp waste-water diluted 200-fold using a LiChrosorb analytical column, mobile phase acetonitrile/acetate buffer (50 mM, pH 4.2) (20:80), peak (1) unknown, peak (2) 5-hydroxymethylfurfural and peak (3) furfural.

necessary to trace low levels of these toxic compounds in biological fluids, and as environmental pollutants.

7.6.4. Amperometric biosensors as detection units in column liquid chromatography and flow injection applied in waste-water and biotechnological samples

Toxic furan aldehydes such as furfural and hydroxymethylfurfural could be determined in fermented industrial waste-water by reversed-phase separations. A LiChrospher silica column was used with an acetonitrile/acetate buffer mobile phase using UV-DAD. The resulting chromatogram is shown in Fig. 7.21 where hydroxymethylfurfural and furfural could be identified by comparing them with the retention times and the UV-DAD spectra, respectively. Several peaks found at the beginning of these chromatograms did not match with the 25 selected phenolic standards and could not be identified. However, examination of their spectra showed they have typical aromatic structures, with two peak maxima at 220 and 280 nm. An example, given in Fig. 7.22, is the spectrum obtained from peak 1 in the chromatogram.

Enzyme-based detection systems have been developed for the determination of aliphatic aldehydes, [108,141–143] and aromatic aldehydes [108,144]. The pyridine nucleotide dependent aldehyde dehydrogenase (EC 1.2.1.3) has been used as the bioactive catalyser in flow systems. Since NAD^+ , the soluble cofactor, is required in the enzymatic reaction, it has to be added to the carrier in FI systems, while it can be added in the mobile phase in single-line CLC systems [108] or added in a reagent flow in the postcolumn mode [144]. Domínguez et al. made an enzyme-kinetic study of this enzyme since it was found that the stability of the aldehyde dehydrogenase when immobilized to a CPG glass support was very poor. Similar deactivation was obtained in batch studies [145]. It was found that the cysteine residues easily undergo an oxidation that affects the active site of the enzyme. The decrease in activity could be stabilized by the addition of a reducing agent, such as mercaptoethanol, to the eluent. By also adding glycine to the eluent and

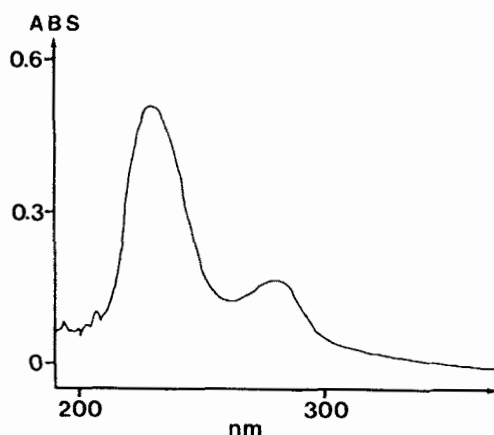


Fig. 7.22. UV-spectrum obtained from peak 1 in Fig. 7.21.

keeping it totally oxygen free, it was possible to maintain the stability of the IMER [146]. This IMER-based detection system was further developed and coupled with amperometric detection using a CME [72,73]. The reaction sequence of the coupled enzymatic and electrochemical reactions is shown in Fig. 7.23. The electrochemistry of NADH oxidation suffers from high overvoltage and side reactions, resulting in electrode fouling [147]. This makes it impossible to use the common electrode materials, glassy carbon, other types of carbonous materials or platinum as stable amperometric detectors for NADH oxidations. The E° of the NAD^+/NADH redox couple is -560 mV versus SCE at pH 7.0; however, a high applied potential around 500 – 700 mV versus SCE (for glassy carbon and platinum electrodes) is necessary for NADH oxidation. This instability in electrode response can be overcome by the use of CME [147–149]. A CME can be described as a deliberate attachment of molecules onto an electrode surface which thereby displays not only its normal properties but also those of the attached molecule. This is done to: (i) reduce the overvoltage of the electrochemical reaction (oxidation or reduction) in order to be able to work at an applied potential around 0 mV, the optimal range for electrochemical detection; (ii) increase the stability; (iii) increase the selectivity of the amperometric oxidation/reduction. Much work has been undertaken to find CMEs for catalytic NADH oxida-

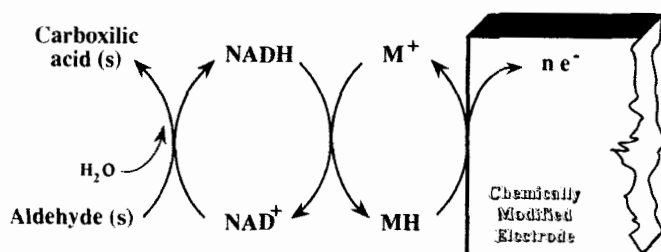


Fig. 7.23 Reaction scheme for the determination of aldehydes.

TABLE 7.1

RELATIVE RESPONSE OF ALIPHATIC AND AROMATIC ALDEHYDES: FOR CONDITIONS SEE FIG. 7.11

Analyte	Relative response (%)
Formaldehyde	10
Acetaldehyde	100
Benzaldehyde	350
Furfural	400
5-Hydroxymethylfurfural	72
Glycolaldehyde	160
Glutaraldehyde	205

tion. From this laboratory, such CMEs have been reported which are based on phenoxazine and phenothiazine derivatives incorporating a positively charged *para*-phenylenediimine functionality [147]. Further improvements have been made by the introduction of the mediator into a polymeric backbone insoluble in water. Electrodes modified with these polymers have an improved long-term stability when used continuously in flow systems for the detection of NADH [150] and when incorporated in enzyme electrodes [151].

The detection system resulted in a combination of enzyme catalysis by the IMER containing aldehyde dehydrogenase and electrocatalysis using a phenoxazine-derivative-CME coupled in the reaction sequence shown in Fig. 7.23. The relative responses of the detection system to other aldehydes are shown in Table 7.1.

This enzyme-based detection system was used in the postcolumn mode in a CLC system. The IMER and the CME were separately optimized in a FI system. The flow-dependence of enzymatically produced NADH in the IMER using this mobile phase showed a dramatic effect for furfural at lower flow rates, between 0.2 ml/min, while other substrates such as acetaldehyde and 5-hydroxymethylfurfural, showed no effect from variation up to 1.5 ml/min (see Fig. 7.24). The electrochemical behaviour of NADH in the mobile phase chosen (see above) was investigated in the potential-range between -400 and +400 mV versus SCE. As can be seen in Fig. 7.25 the resulting cyclic voltammogram reveals optimal oxidation between -200 and +0 mV versus SCE while the background current was found to be at its lowest around 0 mV versus SCE. The oxidation of mercaptoethanol is seen at +200 mV versus SCE. Reversed-phase separation using a PLRP-S column was coupled to the postcolumn detection with a low dead-volume IMER [108]. The chromatographic separation of a fermented waste-water sample spiked with acetaldehyde, 5-hydroxymethylfurfural and furfural is shown in Fig. 7.26. Tyrosinase-based biosensors were also applied for the analysis of phenols in these waste-water samples. A reversed-phase separation system with phosphate buffer/acetonitrile (95:5) as the mobile phase was used with the enzyme electrode in the postcolumn mode (see Fig. 7.20C). The electrode could be operated at around -50 mV versus Ag/AgCl. In most cases, the necessary applied potential of the enzyme electrode is either too high or too low to allow the

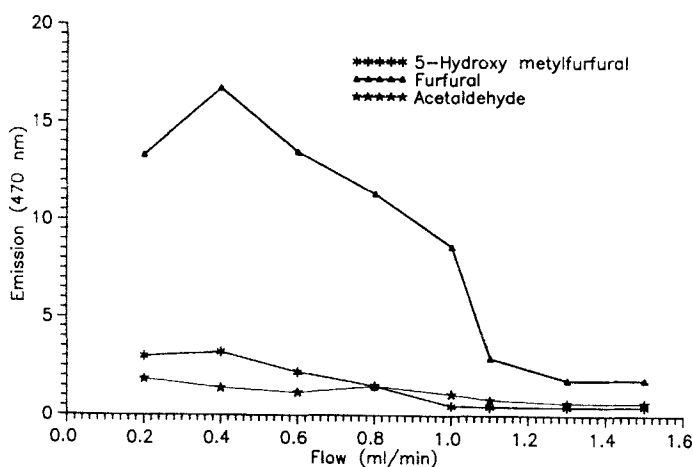


Fig. 7.24. Flow dependence of the aldehyde dehydrogenase IMER in a flow injection set-up using fluorescent detection.

electrochemical reaction to occur without interfering reactions or it results in excessive background currents. The optimal operational potential range for a biosensor to promote sensitive and selective detection should be -200 to 0 mV versus SCE; the background current then switches signs and thus takes its lowest value. Electrochemical reduction of molecular oxygen, and discrimination towards interferents from easily oxidizing or reduc-

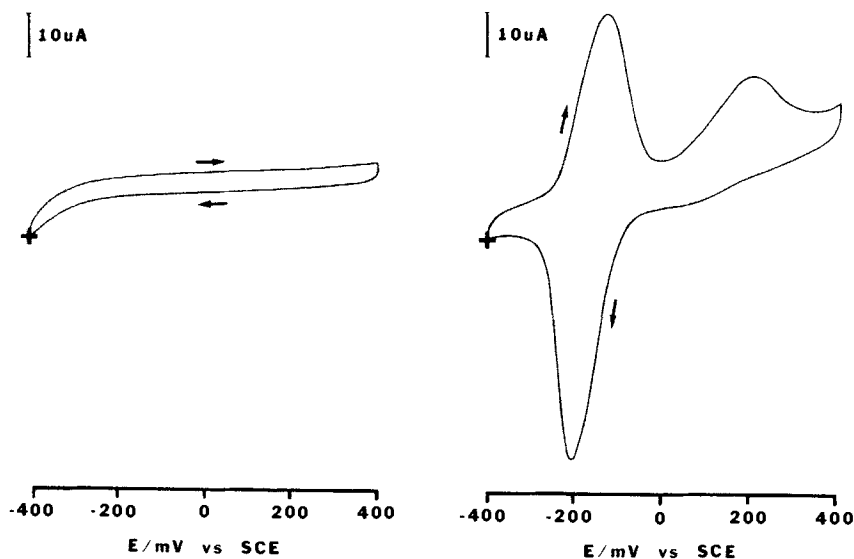


Fig. 7.25. Cyclic voltammograms of (a) naked graphite electrode and (b) same as (a) but modified with a benzophenoxazinyl derivative with an eluent a mixture of methanol/phosphate buffer (0.1 M, pH 7.0) (5:95) with dissolved mercaptoethanol (1 mM), glycine (2 mM).

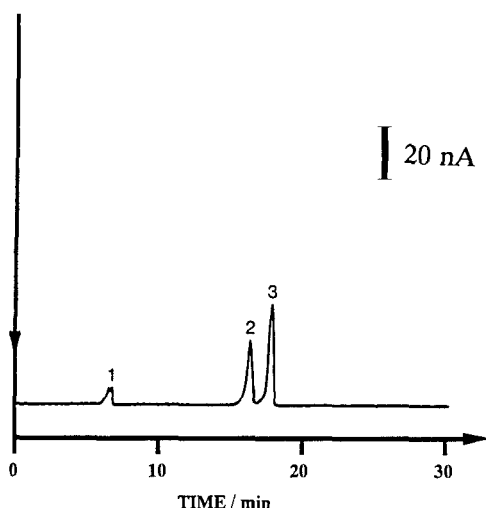


Fig. 7.26. Chromatographic separation of aldehydes using the single line CLC set-up with the IMER in the postcolumn mode. (1) Acetaldehyde, (2) 5-hydroxymethylfurfural and (3) furfural, using a PLRP-S separation column and a mobile phase of a mixture of methanol/phosphate buffer (0.1 M, pH 7.0) (5:95) with dissolved mercaptoethanol (1 mM), glycine (2 mM) and NAD^+ (2 mM)

ing compounds in industrial waste-water samples, are negligible and do not contribute to the response signal [152].

This sensor apparently operated on a direct electron-transfer between the electrode and the tyrosinase at an applied potential of -50 mV versus SCE. The same phenomenon was also seen for a number of other types of biosensors. A reagent-less type of sensor has also been found to work; it uses co-immobilization of a hydrogen peroxide-producing oxidase (e.g. glucose-, alcohol-, lactate-, glutamate-, or L- and D-amino acid oxidases) and a peroxidase [153,155].

Enzyme electrodes were developed which use the immobilization of tyrosinase onto a heat-pretreated carbon electrode surface. Very low detection limits could be obtained in this mode of operation. The high sensitivity is ascribed to the signal-amplification effect taking place on the electrode surface [126]. The enzyme catalyses oxidation of the phenolic compounds, via hydroxylation with molecular oxygen, to catechols and subsequent dehydrogenation to *ortho*-quinones as shown in the reaction sequences of Fig. 7.27. At the electrode surface, the phenolic compound is catalytically oxidized to the quinone which is readily reduced electrochemically back to the phenol thereby making it possible for the phenol to enter a second cycle. This could not be obtained with the IMER (for phenolic catecholamines) because the lack of intimate contact between the enzyme and the electrode.

Tyrosinase enzyme electrode was further developed for inclusion as an enzyme-based detection unit in a CLC separation system for the monitoring of phenolics in waste-water samples from the pulp industry [152]. There is competition between direct electrochemi-

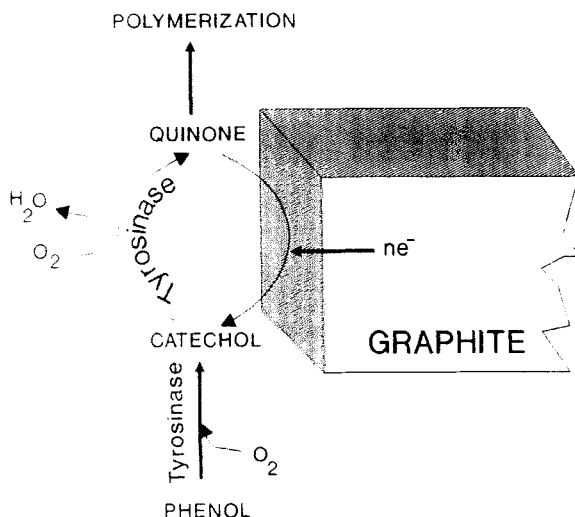


Fig. 7.27. Enzymatic and electrochemical reaction sequences in tyrosinase electrode.

cal reduction of the quinones formed and their polymerization reactions [126]. These competing reactions are currently being studied in our laboratories with the aim of favouring the electrochemical reduction of the enzymatic products.

The sample was used as obtained from a pulp industry in the south of Sweden, diluted 50-fold with distilled water, adjusted to pH 5.0 and subjected to a sterile-membrane filtration step prior to a solid-phase extraction step. Disposable SEP-Pak columns were used, of the silica-based-amino phase type. This solid phase extraction step was used to eliminate some of the brown components present in the almost black waste samples. The clean-up column was conditioned using 2 ml of methanol/water (80:20) to remove impurities from the cartridge, followed by 3 ml of water to remove this solvent mixture, then 2 ml of phosphate buffer (pH 6.0) and ending with 3 ml of water to remove the buffer from the column. Next, 1.5 ml of the waste-water was eluted through the SPE column. Removal of disturbing brown-coloured components such as humic substances and lignin-derived oligomers and polymers, was not complete although the levels were lowered considerably. This was investigated by running UV-spectra before and after the clean-up and recovery studies with phenol and *ortho*-cresol as phenolic standards. Even though a quaternary amine phase (strong anion exchanger) was found to have a higher sorption capacity for the brown-coloured interferences, these SEP-Pak phases showed much lower recovery values for both phenol and *ortho*-cresol. Figure 7.28A shows the separation of the spiked waste-water samples with phenol, *para*-cresol and catechol after treatment by the steps described above. The catechol peak (peak 2) is somewhat tailing for reasons which are still not clear. The blank injection is shown in Fig. 7.28B and no other peaks are found in the chromatogram showing the high selectivity of the tyrosinase biosensor. The same sample was separated and detected by a UV-detector at 270 nm for comparison; these separations are shown in Fig. 7.29. The spiked sample in Fig. 7.29A shows many early-eluting compounds in the chromatogram, but many of the first-eluting compounds

were successfully eliminated in the clean-up step. The separation of the blank (Fig. 7.29B) shows the same separation of early-eluting compounds but also the possible background levels of phenol and catechol present in the sample. These could not be determined by the use of retention data and the UV-detection mode. However, these compounds are not found in the separation of the blank using the enzyme electrode (Fig. 7.28B). Calculation of the concentration of these two possible compounds with UV-detection showed that they would be readily detected by the enzymatic detection mode (especially as the noise of this sensor is very low). The conclusion can therefore be drawn that these peaks are not of phenol or catechol origin.

Other types of enzymes which catalyse the reactions of phenolic substrates are currently under study in our laboratory. In one first attempt, we tried to immobilize laccase (LACC) from *Trichoderma* [156] as follows. Plain graphite-oil paste was prepared by thorough mixing of paraffin oil with heat-treated graphite powder. A plastic syringe was filled with the paste, leaving about 3–4 mm empty on the top to be filled with the enzyme-modified carbon paste to produce the final electrode. The enzyme was immobilized by binding it to the graphitic electrode material by adsorption in the presence of a cationic polymer (polyethyleneimine). After aliquots of enzyme modified paste were filled into the end of the syringe, the end was gently rubbed on glass to produce a flat surface at the tip of the electrode. The final electrodes were mounted in a flow-through amperometric flow-cell of the wall-jet type [157].

The LACC is capable of oxidizing phenolics, as exemplified by the oxidation of phenol in the reaction

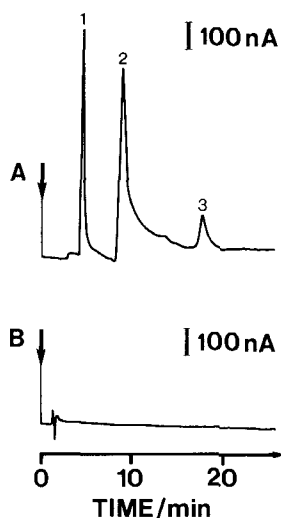


Fig. 7.28. Chromatographic separations of spiked (A) and unspiked (B) waste-water samples after solid phase extraction (for sample handling steps, see text). Chromatographic conditions: analytical column, silica C-18 (LiChrospher); mobile phase, acetonitrile/phosphate buffer (100 mM, pH 6.2) (5:95); injection volume, 20 μ l and applied potential -50 mV versus Ag/AgCl. Peak (1) phenol, peak (2) catechol, and peak (3) *p*-cresol.

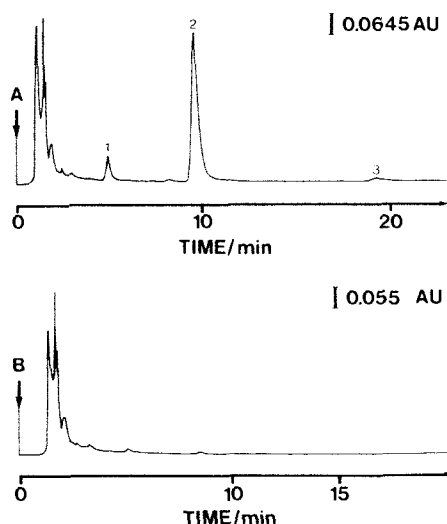
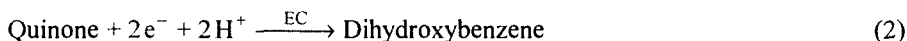
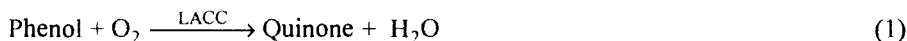


Fig. 7.29. Same conditions as Fig. 7.28, using a diode array detector (HP 1040 M) at 270 nm. Peak (1) phenol, peak (2) catechol, peak (3) *p*-cresol.



The enzymatically produced quinone was next reduced electrochemically to the phenol (reaction 2).

A direct electron-transfer could be obtained in the absence of a mediator. A current response was obtained by injecting of phenol and cresol into a CLC system similar to the one described in Fig. 7.20C with the column omitted. The behaviour of the laccase bio-sensor is depicted in Fig. 7.30 using the flow system described, with 5 ppm phenol and 7 ppm *ortho*-cresol, respectively. However, the catalytic currents obtained with phenol

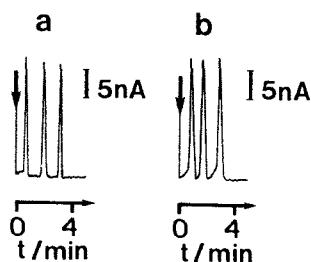


Fig. 7.30. Recordings of laccase modified electrodes, mounted in a flow injection system. Eluent, 0.1 M phosphate buffer (pH 7.0), injection volume 50 μl . (A) phenol 5 ppm and (B) *ortho*-cresol 7 ppm.

and *ortho*-cresol as substrates were low in comparison with those obtained with other enzyme-modified carbon paste electrodes where the oxidases were co-immobilized with peroxidases [153,155]. The catalytic response is also low in comparison with the tyrosinase from mushroom. Thus, this sensor is capable of a direct communication with the electrode in accordance with the tyrosinase enzyme electrode. One of the reasons for this behaviour could be that there are surface-active groups present in the electrode material that interact in an incompatible way. Another way of improving the biosensor performance could make use of various electron acceptors such as 2,4-dichlorophenol, *N*-methylphenazine, benzoquinone or ferrocene derivatives, for the reoxidizing the reduced enzyme back to the oxidized form.

Recently, enzyme-based detection principles have received increasing interest and attention which has led to a programme within the European Community to support their use in environmental control. We will over a 3-year period develop integrated CLC systems, introducing selectivity both before and after the chromatographic separation. The sample-handling steps will be made "on-line" for larger sample volumes, with simultaneous clean-up and trace-enrichment. The separation step will use conventional separation techniques with short analytical columns. The second selective step will be introduced by the use of immobilized enzymes bound to electrode surfaces or on solid supports. The advantages of pre- and postcolumn selectivity will make it possible to analyse and monitor environmental samples of high complexity with high precision and accuracy.

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APPENDIX: LIST OF ABBREVIATIONS

CLC, column liquid chromatography; CPG, controlled pore glass; DAD, diode array detection; EC, electrochemical detection; FI, flow injection; IMER, immobilized enzyme reactor; LACC, laccase; MS, mass spectrometry; NAD^+/NADH , nicotinamide adenine dinucleotide (oxidized and reduced form); NI, negative ionization; PAD, pulsed amperometric detection; PI, positive ionization; RP, reversed phase; SPE, solid phase extraction; TSP, thermospray; TYR, tyrosinase.

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Chapter 8

HPLC methods for the determination of mycotoxins and phycotoxins

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8.1. INTRODUCTION

Mycotoxins are generally considered to be toxic substances produced by certain types of fungi which grow on plants of agricultural importance either before harvest or during storage. Some (such as aflatoxins) are of enough concern that they require regulatory monitoring in plant material destined for human consumption or as animal feed.

Phycotoxins are potentially toxic substances which are produced by marine phytoplankton and which may accumulate in shellfish or finfish through the food chain. Certain phycotoxins such as saxitoxin are acutely toxic to humans and their presence in shellfish has been known for many years. In recent years, it has been recognized that phycotoxins now represent a worldwide concern. It has become extremely important for regulatory agencies throughout the world to ensure that seafood products destined for human consumption are safe. In order to ensure human safety, analytical methods are required that will enable the routine monitoring of food products for both mycotoxins and phycotoxins.

High-performance liquid chromatography (HPLC) has become an extremely important technique for the determination of mycotoxins and phycotoxins in biological samples. Indeed for mycotoxins, extensive research employing HPLC has been reported for many different classes of these substances. Phycotoxins, however, have received much less attention in this regard, mainly due to the difficulty in isolating and identifying the substances causing toxic responses and due to the general lack of standards for most classes of these compounds. However, the need for chemical methods for phycotoxins is becoming increasingly important due to the general trend away from using mammalian bioassays as well as the great increase in culturing fish and shellfish for human consumption thus requiring increased regulatory monitoring. The chemical structures of many mycotoxins and phycotoxins lend themselves to analysis by HPLC rather than gas chromatography or other instrumental techniques. As a result, HPLC has become well exploited for determining these types of compounds.

Research on analytical methods for both mycotoxins and phycotoxins continues at a good pace. Methods for mycotoxins have been the subject of several reviews [1–4] all of which deal with HPLC completely or in part. There have been fewer reviews of analytical methods for phycotoxins [5–7] especially employing HPLC. The aim of this chapter is to present the latest developments in the determination of mycotoxins and phycotoxins by HPLC. Included in this chapter are novel chromatographic systems, sample preparation techniques, the use of chemical derivatization and HPLC-mass spectrometry (MS). Emphasis is placed on the latest research that has been reported.

8.2. MYCOTOXINS

8.2.1. Aflatoxins B₁, B₂, G₁ and G₂

Aflatoxins (see Fig. 8.1 for structure) are secondary metabolites produced by *Aspergillus flavus* and *A. parasiticus* types of molds which grow on many varieties of cereal grains and nuts. These substances have been shown to be both toxic and carcinogenic in many animal species including man [8]. Because of this, active research on sensitive, rapid and reliable methods for their analysis continues. HPLC with fluorescence detection has been used for many years for the determination of aflatoxins [9]. Recent research has focused primarily on improving sensitivity through derivatization reactions or selective detection systems such as HPLC-mass spectrometry. In addition, much work has been reported on improved sample clean-up enabling the detection of lower concentrations of these chemicals.

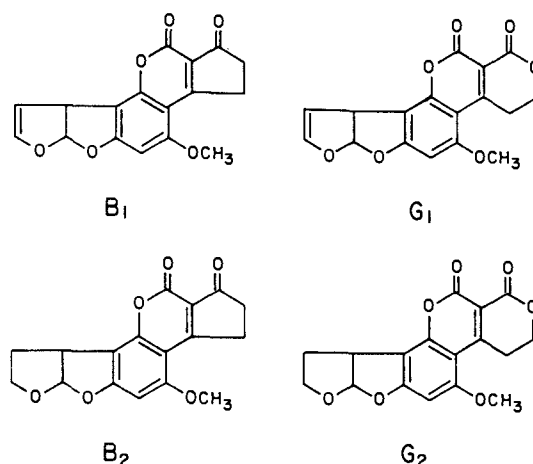


Fig. 8.1. Structures of aflatoxins B_1 , B_2 , G_1 and G_2 .

8.2.1.1. Postcolumn reactions

In the HPLC determination of aflatoxins B_1 , B_2 , G_1 and G_2 using fluorescence detection, only B_2 and G_2 fluoresce with enough intensity in aqueous-based mobile phases to be analytically useful. However, it was observed that reaction of aflatoxins B_1 and G_1 with a variety of reagents such as strong acids [10] or the oxidants, chloramine T [11], bromine [12] or iodine [13–15] led to a significant increase in fluorescence intensity. The iodine reaction has been optimized for enhancement of aflatoxin B_1 [13] and applied to feedstuffs [14,15]. The product of the reaction appears to be an iodide-methoxy addition compound as shown in Fig. 8.2 [16]. The post-column reaction involves mixing the HPLC effluent with saturated aqueous iodine solution and heating at 70°C. The resulting derivatives could be detected in sub-nanogram quantities enabling the detection of ng/g levels in foods and feedstuffs. A modification of this procedure involved the use of a solid phase iodine reservoir [17].

Electrochemically generated bromine has been successfully used for aflatoxin analysis [18,19]. Bromine is generated from bromide present in the mobile phase by passage of the column effluent through an electrochemical cell. The amount of bromine produced can be controlled by the generating current. This approach has several advantages over the iodine postcolumn reaction and is applicable to routine analyses of samples for aflatoxins in the low ng/g range.

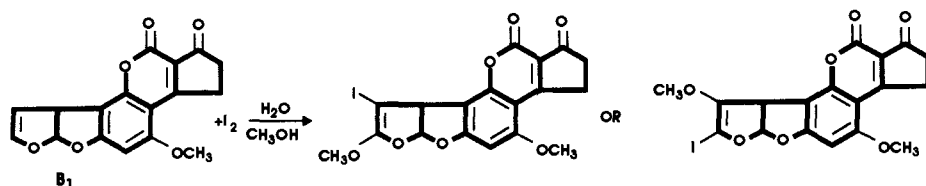


Fig. 8.2. Reaction of aflatoxin B_1 with iodine to yield iodomethoxy addition compounds.

The postcolumn addition of β -cyclodextrin to enhance the fluorescence of B₁ and G₁ was evaluated for the determination of aflatoxins in corn [20]. The fluorescence intensities of aflatoxins B₁ and G₁ were greatly enhanced apparently due to the formation of inclusion complexes with β -cyclodextrin. Sub-nanogram quantities of the toxins could be detected.

8.2.1.2. HPLC-MS

Thermo-spray (TSP) MS coupled to HPLC has been evaluated for the confirmation of aflatoxins and applied to a variety of products [21,22]. Hurst et al. [21] evaluated the technique for the direct confirmation of aflatoxins B₁, G₁, B₂ and G₂ without the need for derivatization. The optimum conditions employed TSP with "filament on" or in the "discharge" ionization mode. Picogram quantities of the aflatoxins could be detected with selected ion monitoring.

In a study of the bisulfite addition compounds of aflatoxin B₁ and G₁ [22], it was found that HPLC-TSP MS could be used to advantage to confirm these substances in a variety of plant extracts. Sodium bisulfite reacts with B₁ and G₁ to form bisulfite adducts across the double bonds of the furofuran ring. Aflatoxin M₁ and aflatoxicol also yield bisulfite adducts. Mass spectrometric responses obtained in the positive ion mode (either with ion-evaporation or "filament on" chemical ionization) were 10–20 fold more intense than with negative ion conditions. Using ion-pair chromatography, the bisulfite adducts of B₁ and G₁ were separable from B₂ and G₂ which were unaffected by the bisulfite treatment. Applications to samples such as corn, milk and liver demonstrated that the method was useful, although for aflatoxin B₁ in corn the bisulfite derivatization method was about five times less sensitive than direct HPLC-TSP MS of the underivatized extracts, which could detect as low as 20 ng/g B₁.

8.2.1.3. Other chromatography and detection work

Tutelyan et al. [23] employed a normal phase HPLC-fluorescence detection procedure with a mobile phase consisting of diethyl ether/methanol/water combinations and obtained good resolution of aflatoxins B₁, B₂, G₁, G₂ and M₁ without pre- or postchromatographic derivatization. They employed a flow-cell packed with silica gel similar to that used elsewhere for normal phase HPLC of these compounds [24]. This method was successfully used to analyse over 4500 plant foodstuffs for aflatoxins. Detection limits were reported to be as low as 100 ng/kg.

Differential-pulse amperometric detection using a dropping mercury electrode was evaluated for the detection of aflatoxins separated by HPLC [25]. It was found that although each of the four aflatoxins (B₁, B₂, G₁, G₂) yielded about the same sensitivity under optimum potential conditions for each individual compound, a compromise potential was required so that the four could be determined together. The detection limits for the individual aflatoxins were similar, being about 5 ng per injection. An advantage of this approach is that B₁ and G₁ do not require derivatization or other special treatment to improve their sensitivity as is required with the fluorometric detection methods.

Capillary micellar electrokinetic chromatography was compared to conventional normal and reverse-phase chromatography for the separation and detection of aflatoxins B₁, B₂, G₁ and G₂ [26]. The technique offers potential in terms of chromatographic efficiency and speed of analysis. However, it has not been applied to quantitative sample analysis on a routine basis.

8.2.1.4. Improved sample clean-up for HPLC

Recent advances in the sample clean-up of extracts for aflatoxin analysis have mainly focused on solid phase extraction (SPE) using small disposable cartridges filled with a variety of adsorbents which selectively retain the aflatoxins or sample co-extractives. Silica gel [27] has been used by several research groups for purification of plant extracts prior to HPLC determination. Hutchins et al. [27] thoroughly evaluated silica SPE cartridges for aflatoxin determination and found that under optimum conditions and using conversion of B₁ and G₁ to their respective hemiacetals, they could detect less than 1 ng/g aflatoxin B₁ in corn with very good repeatability over a wide range of concentrations. The method involved passing a chloroform/hexane extract of corn through a silica SPE cartridge which retained the aflatoxins. The cartridge was rinsed with hexane/chloroform (3:1) before the aflatoxins were eluted with hexane/acetone (1:1). This fraction was clean enough to derivatize and analyse by reverse-phase HPLC with fluorescence detection.

A phenyl-bonded SPE cartridge was successfully employed for the clean-up of groundnut meal extracts [28]. In this case, an aqueous sample extract was mixed with water, acetic acid and a lead acetate solution and then passed through a preconditioned phenyl bonded SPE. The aflatoxins were retained and the cartridge washed with water before the compounds of interest were eluted with a small volume of chloroform. The chloroform fraction was then evaporated to dryness for conversion of B₁ and G₁ to their hemiacetals before reverse-phase HPLC analysis with fluorescence detection. This method could detect low ng/g levels of aflatoxin B₁ and B₂ in groundnut meal.

A novel multifunctional type of SPE cartridge has been evaluated for a variety of plant extracts [29]. The packing material consists of both nonpolar and polar (charged) active sites. The nonpolar sites remove fats and other lipophilic materials while the charged sites (which consist of both dipolar and anion exchange sites) remove proteinaceous substances, carbohydrates and other polar co-extractives. These processes are carried out simultaneously in a single passage of the plant extract through the packing material. The detection limits were less than 0.5 ng/g for aflatoxins B₁, B₂, G₁ and G₂ by reverse-phase HPLC with fluorescence detection.

Immunoaffinity columns are becoming increasingly popular for cleaning up plant extracts for aflatoxin determination by HPLC. There are now a variety of such columns commercially available. They consist of an anti-aflatoxin antibody bound to a gel material packed into a plastic cylinder or cartridge. When an extract is passed through the packing, the aflatoxins bind to the immobilized antibody while other sample co-extractives are washed through the column. The aflatoxins are then recovered by elution of the column with methanol or acetonitrile which liberates them from the antibodies. Because of the extremely selective nature of the clean-up, this type of column has found use for the analysis of aflatoxins by HPLC in a variety of plant products, including nut products and dried

fruit [30–34], and other foods and animal feed [33–35]. Figure 8.3 shows a chromatogram of an extract of dried figs containing low ng/g levels of aflatoxins B₁, B₂ and G₁ which had been cleaned up using immunoaffinity chromatography. As can be seen, the aflatoxins are easily detected at these levels.

A two-column SPE clean-up procedure for aflatoxin B₁ in cattle feed which has successfully passed an interlaboratory study [36] has been automated [37]. The method involves SPE clean-up on a Florisil cartridge followed by a reverse-phase C-18 cartridge and injection of the cleaned up extract into an HPLC system with postcolumn bromination and fluorescence detection. The fully automated procedure compared well with the manual method in terms of repeatability, but the recovery of B₁ at low ng/g levels was only about 40%. The method does appear suitable for screening purposes.

Gel permeation chromatography (GPC) has been evaluated for the clean-up of extracts of cereals and animal feed [38]. The method involves extraction of the aflatoxins with dichloromethane/water (10:1) followed by GPC using dichloromethane/hexane (3:1) as eluent. The fraction containing the aflatoxins is concentrated and then analysed by reverse-phase HPLC. Aflatoxin levels as low as 1 ng/g could be quantitated.

8.2.2. Aflatoxin M₁

Aflatoxin M₁ (Fig. 8.4) is a hydroxylated metabolite of aflatoxin B₁ and is of concern because significant levels of it can occur in milk of animals which have been fed aflatoxin B₁ contaminated feed. Much work on the determination of aflatoxin M₁ in dairy products by a variety of methods have appeared in the literature during the past decade and they

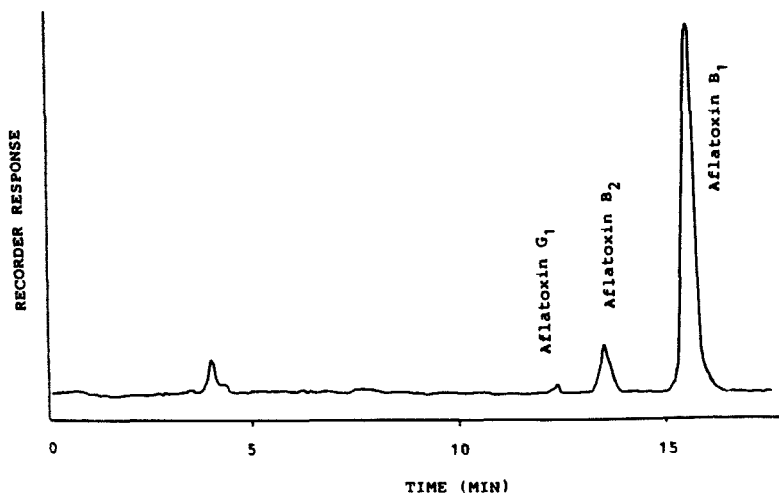


Fig. 8.3. HPLC chromatogram of aflatoxins B₁, B₂ and G₁ in dried figs. Concentrations: 11.5 ng/g B₁, 1.1 ng/g B₂ and 0.6 ng/g G₁. Reverse-phase chromatography with postcolumn iodination and fluorescence detection. (Reproduced from ref. 31 with permission of Taylor & Francis, London, UK.)

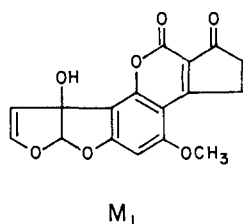


Fig. 8.4. Structure of aflatoxin M_1 .

have been reviewed [39]. Since then new developments continue to be reported for aflatoxin M_1 determination in dairy products.

Aflatoxin M_1 , like B_1 and G_1 , has been found to fluoresce more intensely after derivatization. The reaction conditions for the conversion of M_1 to M_{2a} using trifluoroacetic acid (TFA) have been studied in detail [40]. However, M_1 does itself fluoresce enough to enable its direct determination in dairy products depending upon the effectiveness of the sample clean-up.

Immunoaffinity column clean-up of extracts of dairy products for HPLC analysis has been evaluated using both off-line [41,42] and on-line [43–45] procedures. The off-line procedure for milk involved only a centrifugation step to remove fat before the milk was passed through the immunoaffinity columns [41]. More than a litre of milk could be passed through a single column without difficulty. HPLC analysis involved reverse-phase chromatography with direct fluorescence detection. The affinity column clean-up was extremely effective, resulting in detection limits of about 50 pg/l. Application of the same type of affinity column to cheese samples required extraction and liquid-liquid partitioning before the sample extract could be loaded onto the column [42]. Although not as simple as the affinity column clean-up for liquid or powdered milks, the method was superior to other procedures for M_1 in cheese. The detection limits were about 5 ng/kg using the same HPLC-fluorescence detection system as for milk.

The on-line affinity column clean-up-HPLC analysis involved column switching first to load and wash the affinity column before elution and second, to transfer the M_1 fraction to a reverse-phase precolumn for collection and preconcentration of the toxin before switching to the analytical column [43–45]. One of the main difficulties with this approach was that after a few sample analyses, the affinity column lost its effectiveness. An on-line dialysis unit has been evaluated as an additional clean-up before the affinity column [45]. Although repeatability of the fully automated system was good, the overall recovery was extremely low (about 6%). Detection limits were reported to be about 10 ng/l.

Other automated systems incorporating column switching for on-line dialysis followed by reverse-phase enrichment of aflatoxin M_1 and HPLC with fluorescence detection have been evaluated for milk samples [46,47]. Recoveries with the dialysis procedure were about 50%. Detection limits were in the range of 20–50 ng/l.

An automated method incorporating a laboratory robot has been developed for aflatoxin M_1 in milk [48]. The robot operated an SPE extraction station which involved sample application, washing and elution. The fraction containing aflatoxin M_1 was transferred to an automated HPLC system, which included an on-line preconcentration unit, for de-

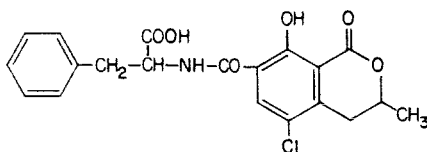


Fig. 8.5. Structure of ochratoxin A.

termination using reverse-phase chromatography with fluorescence detection. The system could handle 18 samples in an 8-h work period. The method could measure $0.1 \mu\text{g/l}$ aflatoxin M_1 in milk.

8.2.3. Ochratoxin A

Ochratoxin A (structure, Fig. 8.5) is a nephrotoxic and carcinogenic mycotoxin that is highly toxic to several animal species. It is produced by fungi of the *Aspergillus* and *Penicillium* genera which infect cereal grains and other plants used as human food or animal feed. Because of this, much research has been reported in the literature on method development, particularly by HPLC, so that surveillance data could be generated on the presence of ochratoxin A in food and animal feed. Also, since animals can retain detectable amounts in their blood and certain tissues, there is a need to have methods capable of monitoring animal products as well.

Analytical methods (including TLC, HPLC, spectrophotometry and immunoassay) for ochratoxin A have been recently reviewed [49]. Reverse-phase HPLC with an acidified mobile phase and fluorescence detection has been employed for the determination of ochratoxin A in cereal grains [50,51], animal feed [51], kidney [50] and human urine [52]. These methods employed an initial organic solvent extraction followed by SPE or open column chromatography clean-up before analysis by HPLC. This approach successfully passed an interlaboratory collaborative study [50]. Detection limits were in the low ng/g range for the cereals and kidney and in the ng/l range for human urine.

Ion-pair HPLC has been successfully used for determination of ochratoxin A in coffee products [53] and human plasma [54]. In this method, the mobile phase was made basic (pH 7.5–9) and low millimolar concentrations of ion pairing agents such as cetyltrimethyl ammonium bromide or tetrabutyl ammonium bromide were added. Detection limits for coffee products were in the sub-ng/g range when affinity chromatography clean-up was employed [53]. The detection limits for the ion-pair HPLC plasma method were 0.02 ng/ml [54].

Immunoaffinity column clean-up has been shown to provide very clean extracts for ochratoxin A analysis by HPLC. In a comparison of affinity chromatography and reverse-phase SPE chromatography for clean-up of extracts of the coffee products mentioned above, the affinity chromatography clean-up yielded about a four fold improvement in detection limits. Affinity chromatography has been employed in an automated HPLC technique for ochratoxin A in cereal and animal products [55]. The affinity column clean-up

was carried out either manually or with a commercial automated sample preparation system similar to that used for aflatoxins [32]. The authors found that the immunoaffinity approach was significantly faster than methods employing adsorption type chromatographic clean-up. The method was particularly attractive since the selectivity of immunoaffinity clean-up led to very clean chromatograms and unambiguous identification and quantitation of ochratoxin A at sub-ng/g levels in cereals and pig kidney. Figure 8.6 shows chromatograms of extracts of wheat and pig kidney containing low ng/g levels of the toxin. The HPLC separation involved reverse-phase chromatography with an acidified mobile phase and fluorescence detection (333 nm excitation, 477 nm emission).

To confirm positive findings of ochratoxin A, several researchers have used chemical derivatization [52–54]. The reactions involved esterification of the carboxylic acid moiety of the toxin using either H_2SO_4 or BF_3 catalysed reaction with methanol, ethanol or propanol. The resulting esters were determined using the same reverse-phase HPLC system as for unesterified toxin and were more strongly retained on reverse-phase columns. HPLC-MS has been evaluated for confirmation of ochratoxin A in barley extracts [56]. In this work, a direct liquid introduction (DLI) interface was employed with a quadrupole mass spectrometer. The HPLC effluent, consisting of aqueous acetonitrile acidified with formic acid, was split so that only 2.5% of the mobile phase entered the DLI interface. Using negative ion chemical ionization and monitoring at m/z 403 (M^-), sub-ng/g levels of ochratoxin A were detectable.

8.2.4. Fumonisin

The fumonisins (structures, Fig. 8.7) are a group of mycotoxins produced by *Fusarium moniliforme* Sheldon, a common fungal contaminant of corn. Because of their toxicity and carcinogenicity, there is a need for sensitive, accurate methods for their analysis in corn products destined for human consumption. A number of HPLC methods for fumonisins have been reported in the literature [57–60]. Since fumonisins have no analytically useful UV absorption or fluorescence, they must be derivatized to products which are detectable using standard HPLC detectors. Thus the methods reported to date involve UV [57] or fluorescence [57–61] derivatization with reagents such as maleyl anhydride [57], *ortho*-phthalaldehyde (OPA) [58,61], fluorescamine [57], 4-fluoro-7-nitrobenzofurazan

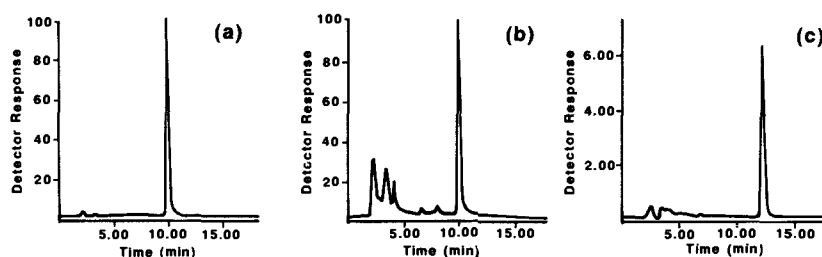


Fig. 8.6. HPLC chromatograms of ochratoxin A. (a) Standard equivalent to 20 ng/g. (b) Naturally contaminated wheat containing 13.7 ng/g. (c) Pig kidney spiked with 10 ng/g. (Reproduced from ref. 55 with permission of Elsevier, Amsterdam.)

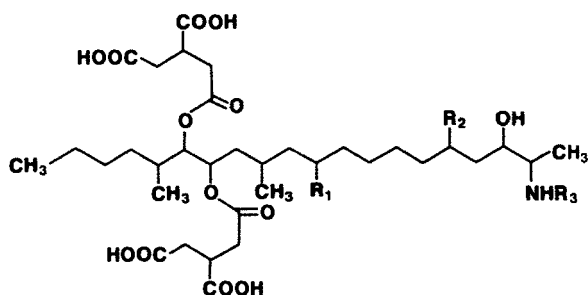


Fig. 8.7. Structures of fumonisin B₁ (R₁ = R₂ = OH, R₃ = H), fumonisin B₂ (R₁ = H, R₂ = OH, R₃ = H), fumonisin B₃ (R₁ = OH, R₂ = R₃ = H), fumonisin B₄ (R₁ = R₂ = R₃ = H), fumonisin A₁ (R₁ = R₂ = OH, R₃ = CH₃CO) and fumonisin A₂ (R₁ = H, R₂ = OH, R₃ = CH₃CO).

(NBDF) [59,60] and naphthalene-2,3-dicarboxaldehyde (NDA) [59]. The methods employing OPA, NBDF and NDA appear to offer the most in terms of method simplicity and detection limits. These methods used methanol/water for extraction of corn samples followed by strong anion exchange SPE clean-up of the extracts. The fumonisins were eluted with methanol/acetic acid and the fraction evaporated to dryness before derivatization. Separations were carried out by reverse-phase chromatography using slightly acidified

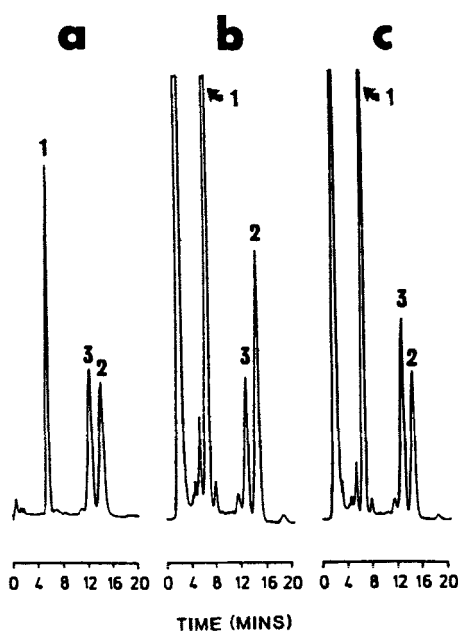


Fig. 8.8. Chromatograms of (a) OPA derivatives of 50 ng of fumonisin B₁ (1), B₂ (2), and B₃ (3); (b) derivatized extract of corn-based feed sample (8 µg/g B₁, 4.1 µg/g B₂ and 2.7 µg/g B₃); (c) as in (b) but spiked with standard B₃. (Reproduced from ref. 61 with permission of AOAC International, Arlington, VA.)

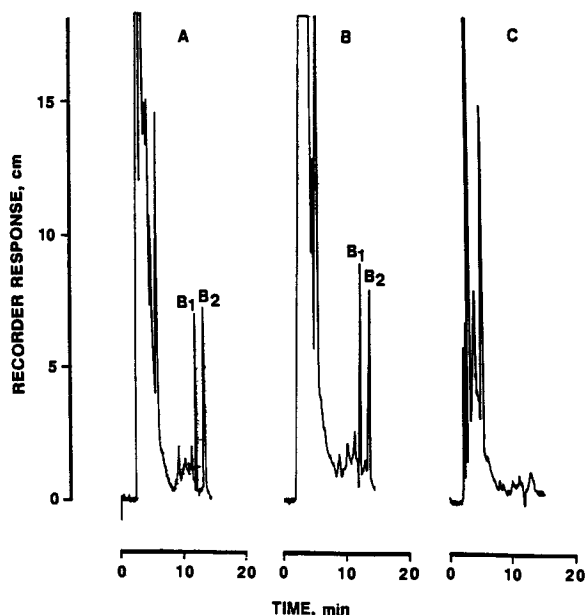


Fig. 8.9. Chromatograms of derivatized extracts of (A) corn meal spiked with $2.5 \mu\text{g/g}$ each of fumonisins B_1 and B_2 . (B) spiked corn meal kept at 22°C for 1 h, and (C) spiked corn meal after heating at 220°C for 25 min. (Reproduced from ref. 60 with permission of AOAC International, Arlington, VA.)

mobile phases (pH 3.5–5). Detection limits using OPA as the fluorescent derivatization reagent were about 50 ng/g for fumonisin B_1 , and about 100 ng/g for fumonisin B_2 . The NBDF and NDA methods could detect fumonisins B_1 and B_2 in the 100 ng/g range [59]. Figure 8.8 shows chromatograms of an extract of a corn-based feed sample containing low $\mu\text{g/g}$ levels of fumonisins B_1 , B_2 and B_3 after conversion to the OPA derivatives. Figure 8.9 shows examples using NBDF as derivatizing reagent.

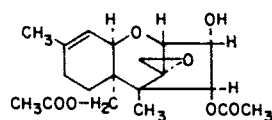
There has been one report of using HPLC-MS for the confirmation of fumonisin B_1 in culture by *Alternaria alternata* f. sp. *lycopersici* [62]. However, no application to trace residue analysis was included.

8.2.5. Trichothecenes

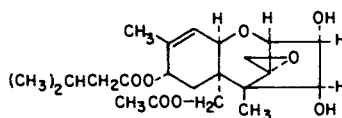
Trichothecenes (structures, Fig. 8.10) are mycotoxins produced by *Fusarium* spp. and other types of fungi which can contaminate grains and other plants. There has been a large amount of work reported in the literature on the analysis of these substances by various chromatographic means, including HPLC. This area has been reviewed [63].

Recent HPLC methods for trichothecenes have involved attempts to improve sensitivity by evaluating a variety of derivatization reagents to make the mycotoxins more detectable by fluorescence or UV absorption. Reagents such as coumarin-3-carbonyl chloride [64] and anthracene-9-carbonyl chloride [65] have been successfully studied for several

TYPE A

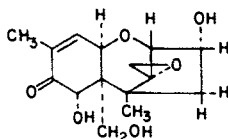


DIACETOXYSCIRPENOL

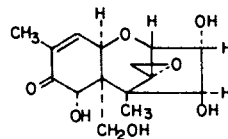


HT-2 TOXIN

TYPE B



DEOXYNIVALENOL



NIVALENOL

Fig. 8.10. Structures of four trichothecene mycotoxins.

trichothecenes. These reagents react with the hydroxyl substituents to form fluorescent products which are separable by reverse-phase HPLC. Low nanogram quantities of T-2 toxin, for example could be detected. These methods have not, however, been evaluated for routine detection in plant materials.

Diphenylindenone sulfonyl (Dis) esters of several trichothecenes including T-2 toxin, HT-2 toxin, diacetoxyscirpenol, T-2 triol, T-2 tetraol and iso-HT-2 toxin were prepared for both TLC and HPLC analysis [66]. For HPLC, UV detection at 278 nm was employed. However, the minimum detectable quantities per injection were 30–50 ng which is not sensitive enough for residue determinations in contaminated grains or other plants. The authors found the method particularly suitable for *in vivo* pharmacokinetic studies involving T-2 triol and T-2 tetraol.

Postcolumn treatment of the effluent by means of photolysis [67] or with alkali followed by reaction with methyl acetoacetate/ammonium acetate [68] have been evaluated for deoxynivalenol (DON), nivalenol and fusarenon-X. The photolysis method involves passing the HPLC effluent through a postcolumn UV irradiation unit which converts the trichothecenes to oxidizable products that can be detected by oxidative amperometric detection. As low as 1–2 ng of DON could be detected. The advantage of this type of derivatization is that no postcolumn reagent addition is required. The alkali-methyl acetoacetate reaction appears to be somewhat less sensitive and considerably more complex requiring two reagent pumps and reaction coils. However, with suitable clean-up DON and nivalenol could be detected in corn, wheat and barley at 0.05–1 µg/g concentration levels.

Direct reductive electrochemical detection (–1.4 V) has been studied for application to the HPLC analysis of DON in corn, rice and wheat products [69]. Sub-nanogram quantities could be detected and, with the extraction and clean-up procedure employed, sub-µg/g levels of DON could be quantitated in grain samples. The method, however, was not applicable to the type A toxins (see Fig. 8.10) since they were electrochemically inactive under the conditions used.

An HPLC column-switching technique has been developed for the determination of DON in maize and corn silage [70]. It involved heart-cutting from one reverse-phase C-18 column to a second. The method could detect as low as $0.2 \mu\text{g/g}$ of DON using UV detection at 215 nm.

HPLC-MS has been shown to offer potential for trichothecene determination: TSP-MS, dynamic fast atom bombardment (FAB)-MS and plasma-spray-MS have all been evaluated for a variety of trichothecene analogues [71–75]. The TSP mass spectra exhibit practically no fragmentation showing only an abundant ammonium adduct ion. Plasma-spray, involving a somewhat more energetic ionization process than thermo-spray produces some fragment ions in addition to the same ammonium adduct ion as observed with thermo-spray. Dynamic FAB yielded numerous fragment ions [74]. All three techniques appear suitable for monitoring trichothecenes in plant or animal tissues without the need for derivatization. However, for structural identification, the dynamic FAB technique would be preferred because of the additional fragmentation obtained. HPLC-MS has not been studied extensively for residue analysis although the application of TSP-MS to the detection of several toxins (DON, T-2 toxin and diacetoxyscirpenol) in porcine plasma and urine has been reported [75]. Detection limits were about 1 ng per injection for the trichothecenes examined.

8.2.6. Zearalenone

Zearalenone (structure, Fig. 8.11) and its metabolites, α and β -zearalenol are biologically active mycotoxins possessing estrogenic activity. There is also some evidence for the carcinogenicity of zearalenone. This mycotoxin is produced by *Fusarium graminearum* and several other species of *Fusarium* fungi which can infect a variety of agricultural crops, particularly corn and other grains.

Recent HPLC methods for zearalenone determination have employed reverse-phase chromatography with direct fluorescence detection [76–78]. These methods used several

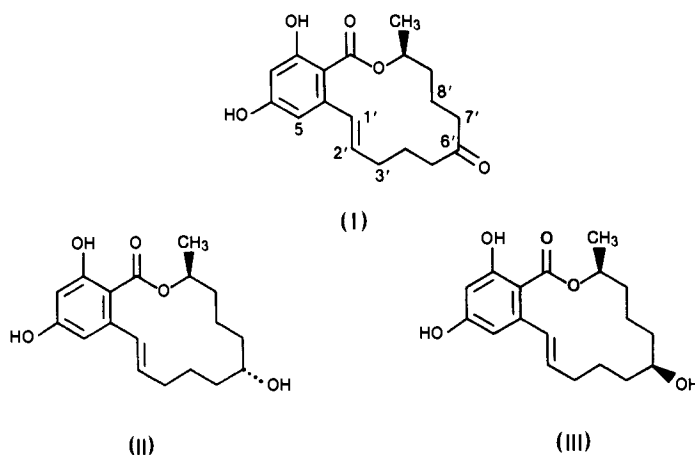


Fig. 8.11. Chemical structures of zearalenone (I), α -zearalenol (II) and β -zearalenol (III).

different sample clean-up techniques. Liquid-liquid partitioning was used to purify aqueous extracts of biological fluids (milk, blood, urine, bile) from cows or swine [76]. Detection limits for zearalenone and the two zearalenols were in the low ng/ml range. Solid phase extraction using an aminopropyl column was found to be useful for clean-up of milk after an initial extraction with basic acetonitrile and partitioning into dichloromethane [77]. The partitioning was speeded up considerably using a hydrophilic matrix to absorb the aqueous phase after removal of acetonitrile by rotary vacuum evaporation. The clean-up enabled the detection of zearalenone and α -zearalenol in milk at levels as low as 0.2 ng/ml and for β -zearalenol, down to 2 ng/ml. GPC was used to purify chloroform extracts of a variety of cereal and mixed animal feed samples for the determination of zearalenone [78]. Although useful for certain grains, the extracts of mixed feeds required additional clean-up using SPE silica columns. The detection limit using HPLC with an amino-bonded phase and fluorescence detection at 280 nm (excitation) and 470 nm (emission) was about 2 ng/g.

The HPLC fluorescence detection of zearalenone in cereal extracts was modified by adding aluminum chloride solution to the column effluent in a heated postcolumn reactor [79]. The reaction with zearalenone resulted in a five-fold increase in fluorescence response. The nature of the reaction involved is still unknown. The authors found that this was particularly useful for determining low levels of zearalenone in cereals. However, in some cases, the matrix background in the chromatograms also increased substantially with the addition of aluminum chloride.

Electrochemical detection has been evaluated for the detection of zearalenone and zearalenol in corn [80]. The method involved chloroform extraction, liquid-liquid partition and reverse-phase HPLC with detection at +0.95 V using a glassy carbon working electrode. This system was capable of detecting as low as 20 pg per injection of the mycotoxins. Detection limits in corn were in the low ng/g range.

8.2.7. Cyclopiazonic acid

α -Cyclopiazonic acid (structure, Fig. 8.12) is a toxic mycotoxin produced by certain species of *Aspergillus* and *Penicillium*. It has been found in several plant products, such as corn and peanuts, as well as in *Penicillium* processed cheese. It is also known to accumulate in certain animal tissues as a result of the consumption of contaminated feed. Thus

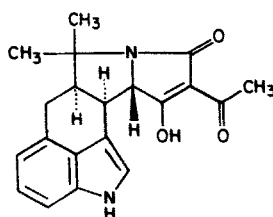


Fig. 8.12. Structure of cyclopiazonic acid.

there is a need to monitor for the compound in plant and animal products which may be consumed by humans.

Several different HPLC methods have been evaluated for the determination of cyclopi-azonic acid in corn, peanuts, poultry tissue and culture media. These include ligand-exchange chromatography [81–83], normal phase chromatography [84] and ion exchange chromatography using an amino-bonded phase [85]. All methods employed UV absorbance detection at a wavelength between 279 and 284 nm or 225 nm [85]. The ligand-exchange methods made use of zinc acetate or zinc sulfate (as a chelating agent) to improve the peak shape when employing reverse-phase columns and aqueous mobile phases. The extraction and clean-up procedures mostly involved solvent extraction and liquid-liquid partition followed by SPE clean-up on silica. Quantitation limits were in the 50–100 ng/g range for corn, peanuts and poultry tissue using the ligand exchange systems [82,83].

8.2.8. Miscellaneous mycotoxins

HPLC has been used for the determination of a variety of mycotoxins as demonstrated above. The technique has also been applied to other types of mycotoxins although on a more limited scale due to both the availability of better methods or to the fact that many of these compounds are not of much interest because of their limited presence in plant or animal products destined for consumption as food or animal feed.

Alternaria mycotoxins, including alternariol, alternariol monomethyl ether, altenuene and tenuazonic acid have been determined by HPLC using several different extraction procedures and chromatographic systems. The separations involved ligand exchange, anion exchange and ion pair chromatography [86,87], reverse-phase [88] and normal phase [89] chromatography. All employed UV detection either around 280 nm or 340 nm. The methods were applied to laboratory cultures [86–88], rice leaves [86], tomatoes [89] and apples [89]. Electrochemical detection was found to be useful for the detection of alternatoxins separated by reverse-phase HPLC [90]. This procedure involved dual in series electrodes operating in the redox mode where the toxins were oxidized (+1.0 V) by the first electrode, then the products detected at the second electrode at a negative potential (−0.1 V). The approach could detect several of the toxins at sub- $\mu\text{g/g}$ levels in maize, rice and tomatoes infected by *Alternaria alternata*.

Citreoviridin is a neurotoxic mycotoxin which may be present in corn or rice infected with certain *Penicillium* fungi such as *P. citreo-viride*. The toxin has been determined in corn and rice using organic solvent extraction, SPE clean-up on silica and amino columns and normal phase HPLC with fluorescence detection (388 nm, excitation and 480 nm emission) [91]. Minimum detectable concentrations were about 2 ng/g for corn.

Citrinin, a mycotoxin produced by several *Aspergillus* and *Penicillium* species has been determined in cereals using normal phase [92] or reverse-phase ion pair [93] HPLC with fluorescence detection. Ion-pair chromatography was required for the reverse-phase system to improve peak shape and column efficiency. The normal phase system employed a novel acid-buffered silica column where the column was conditioned with aqueous citrate, then it was completely dried before passing the hexane-chloroform mobile phase through. Good peak shape and sensitivity were obtained using both methods either in fun-

gal fermentations [93] or in cereals [92]. Low to sub ng/g levels of citrinin could be detected [92].

Normal phase HPLC with UV absorption has been used for the determination of the *Fusarium moniliforme* produced mycotoxin, fusarin C, in submerged cultures [94]. The method employed semi-preparative normal phase HPLC to purify the culture extract followed by analytical HPLC with UV detection for quantitation. Three isomers were observed in the extracts due in part to decomposition under UV light. HPLC has been used in combination with an ELISA immunoassay technique for the determination of another *Fusarium* mycotoxin, fusarochromanone, in barley, wheat and in cultures of rice and corn [95]. After initial extraction with methanol, the mycotoxin was isolated by reverse-phase HPLC and then acetylated and determined by ELISA. The detection limit was about 5 ng/g in barley and wheat compared to a detection limit of about 20 ng/g when ELISA was used without the HPLC purification. Moniliformin is a mycotoxin produced by a variety of *Fusarium* species. An HPLC method has been developed for its determination in cereals [96]. The technique involved extraction with acetonitrile/water followed by SPE clean-up on a combination of reverse-phase and strong anion exchange materials. The extracts were analysed by ion-pair HPLC with UV detection at 229 nm. The detection limit was about 50 ng/g.

Gliotoxin, a fungal toxin produced by *Aspergillus fumigatus* Fresenius and other *Aspergillus* species, has been determined in rice cultures by reverse-phase HPLC with UV detection at 254 nm [97]. The chloroform extracts were cleaned up using GPC before HPLC determination. As low as 0.67 µg/g of gliotoxin could be determined in rice cultures.

Phomopsin A is a toxin produced by *Phomopsis leptostromiformis*, a fungus which can infect sweet narrow-leaved lupins often used as summer feed for sheep in Australia. A method employing HPLC has been evaluated for detection of phomopsin A in lupin stubble [98]. The toxin is extracted with methanol/water, then subjected to liquid-liquid partition, followed by column clean-up on Amberlite XAD-2 and cation exchange chromatography. The final purified extract is analysed by reverse-phase HPLC with UV detection at 280 nm. As low as 0.5 µg/g could be detected.

Tremorgenic mycotoxins such as the paspalitrem and lolitrem groups are produced by several types of fungi including *Aspergillus*, *Claviceps* and *Penicillium*. Several paspalitrems have been determined (in cultures) by normal phase HPLC with diode array UV detection [99]. Lolitrem B (the major lolitrem toxin) has been determined in perennial rye grass by HPLC with fluorescence detection [100]. Fluorescence was found to be much more sensitive and selective than UV absorption for detection of lolitrem B in the rye grass samples.

Ergot alkaloids are produced by the fungus *Claviceps purpurea* which can infect a variety of grains, particularly rye and the animal feed grass, tall fescue. Recent HPLC methods for ergot alkaloids include minor modifications [101,102] of the reverse-phase method first reported by Scott and Lawrence [103]. These methods were applied to the analysis of endophyte-infected tall fescue [101,102]. They made use of reverse-phase gradient HPLC with a slightly alkaline mobile phase and fluorescence detection. Detection limits for the various alkaloids were in the 50–200 ng/g range. A gradient ion-pair HPLC system using fluorescence detection was employed in a survey of cereals for a

number of ergot alkaloids [104]. The ion pairing mobile phase improved peak resolution and led to a much longer column life compared to the alkaline mobile phase used earlier [103]. Detection limits were in the low ng/g range.

8.2.9. Multimycotoxin methods

A number of methods have been developed for the determination of a variety of mycotoxins in a single analytical scheme. Such methods are particularly useful because of the possibility of having several different mycotoxins present at the same time on certain crops. For example, a method for the extraction and detection of aflatoxins, ochratoxin A and zearalenone in grains, oilseeds and animal feeds using on-line sample clean-up and HPLC with postcolumn iodine treatment has been reported [105]. A method for detecting these same mycotoxins plus vomitoxin and secalonin acid D in grain dust was developed which used TLC for the detection of the aflatoxins and ochratoxin A and reverse-phase HPLC with UV detection for the remainder [106]. A multimycotoxin method for patulin, penicillic acid, zearalenone and sterigmatocystin was developed for analysis of cocoa beans [107]. This method also used HPLC as the determinative step with simultaneous dual channel UV detection at 245 nm and 280 nm. All of these methods could detect the mycotoxins at levels of concern.

Diode array UV absorbance detection has proven to be very useful for multimycotoxin screening by HPLC. Several studies have shown the potential of the technique for monitoring a wide variety of toxins. Frisvad and Thrane [108] reported on the HPLC-diode array UV characteristics of 182 mycotoxins and other fungal metabolites. They used an alkylphenone retention index system for normalizing retention data. Similarly Paterson et al. [109] and Kuronen [110] evaluated HPLC with diode array detection for an extensive number of mycotoxins and fungal metabolites. This work has provided valuable information on the chromatography and absorption characteristics of many mycotoxins. Although it is unlikely that any one multimycotoxin procedure will be practical for the routine determination of such a wide variety of mycotoxins at levels of concern, these data indicate that in many cases multimycotoxin determination of selected toxins is very feasible.

HPLC-TSP MS has been used for the determination of a number of *Fusarium* mycotoxins [75] as well as a combination of toxins including several trichothecenes, patulin, zearalenone and ochratoxin A in a single chromatographic run [111]. In the latter case detection limits for the mycotoxins were in the 1–40 ng/g range in grain samples. HPLC-MS continues to demonstrate itself as a technique with much potential for routine determination and confirmation of mycotoxins in biological samples.

8.3. PHYCOTOXINS

8.3.1. Paralytic shellfish poisons

Toxins associated with paralytic shellfish poison (PSP) represent a group of highly polar water soluble compounds with structures as shown in Fig. 8.13. The most commonly used method at present for PSP toxin determination is the mouse bioassay [112]. It

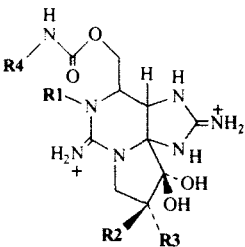
	Substituents				Toxin
	R1	R2	R3	R4	
	H	H	H	H	Saxitoxin (SAX)
	H	H	H	SO ₃	B1
	H	H	OSO ₃	H	Gonyautoxin 2 (GTX 2)
	H	H	OSO ₃	SO ₃	C1
	H	OSO ₃	H	H	Gonyautoxin 3 (GTX 3)
	H	OSO ₃	H	SO ₃	C2
	OH	H	H	H	Neosaxitoxin (NEO)
	OH	H	H	SO ₃	B2
	OH	H	OSO ₃	H	Gonyautoxin 1 (GTX 1)
	OH	H	OSO ₃	SO ₃	C3
	OH	OSO ₃	H	H	Gonyautoxin 4 (GTX 4)
	OH	OSO ₃	H	SO ₃	C4

Fig. 8.13. Structures of 12 PSP toxins.

is a method that measures total toxicity of shellfish extracts and, when set up properly, is an efficient means of monitoring the safety of shellfish. However, the method requires a constant supply of mice and maintenance facilities that are not available in most analytical chemistry laboratories. As a result, research on chemical methods for PSP toxin analysis continues to be pursued.

8.3.1.1. Postcolumn oxidation reactions

The most successful of the chemical methods to date involves HPLC with postcolumn oxidation and fluorescence detection [113–115]. Figure 8.14 illustrates a typical arrangement of equipment for PSP determination using the technique. The separation of the in-

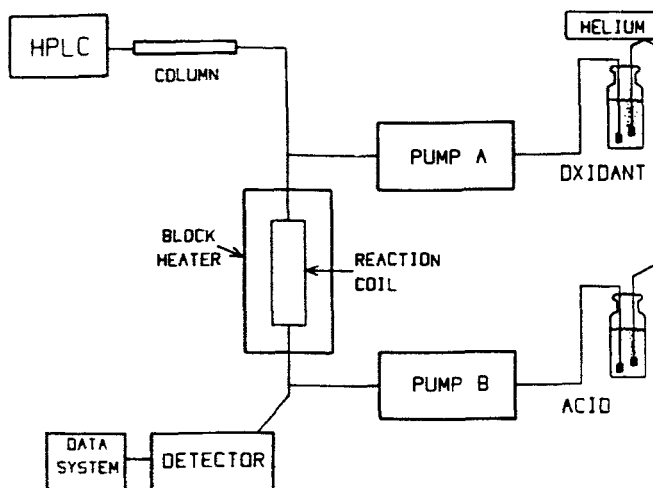


Fig. 8.14. Schematic diagram of the apparatus for postcolumn oxidation of PSP toxins. (Reproduced from ref. 114 with permission of Elsevier, Amsterdam.)

dividual PSP analogues is accomplished using gradient elution ion-pair chromatography on a styrene-divinylbenzene copolymer column (PRP-1, Hamilton). The toxins are detected by fluorescence after conversion to purine derivatives by oxidation with periodate at weakly basic pH. After the oxidation, the reaction stream is adjusted to about pH 5 to optimize fluorescence yield. Figure 8.15 shows a typical separation of a standard mixture of PSP toxins as well as chromatograms obtained with contaminated clams and mussels.

The oxidation reaction is based on earlier work [116] where PSP toxins were oxidized with H_2O_2 to yield fluorescent products and the total amount of fluorescence produced was used as an estimate of PSP concentration. However, since then it was found that the N-1 hydroxy containing toxins are poorly oxidized with H_2O_2 [113], thus use of this reagent alone can seriously underestimate the true concentration of PSP in unknown extracts. Periodate was found to produce fluorescent products with all PSP toxins although the yields of individual analogues vary significantly. Thus, for a chemical method, it is essential to separate the analogues and quantitate them individually. Continuous flow autoanalysers without a chromatographic separation step have been investigated [117, 118]. However, these have found little use due to the problems mentioned above.

The major problem with the postcolumn oxidation method described above is a technological one. The system has been found to be very sensitive to slight changes in temperature, pH and chromatographic conditions, making it difficult to optimize and maintain both chromatographic separation and sensitivity for all toxins of concern. In addition, the method cannot determine the four C-toxins which, although much less toxic than saxitoxin, are of interest since they can be converted to their more toxic analogues, the gon-yautoxins (GTX1–GTX4) under acidic conditions (pH 1–2) [119]. The postcolumn method is much more suited to monitoring for PSP contamination on an on-going basis rather than to being set up for determinations on an occasional basis.

Oshima et al. [120] substantially modified the postcolumn method developed by Sullivan and co-workers [113,114]. The significant changes made were in the chromatog-

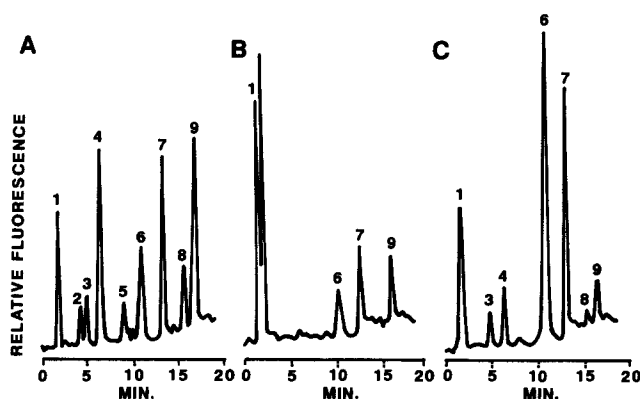


Fig. 8.15. Chromatograms of PSP toxins. 1 = C1 and C2, 2 = B2, 3 = GTX4, 4 = GTX1, 5 = B1, 6 = GTX3, 7 = GTX2, 8 = neosaxitoxin, 9 = saxitoxin. (A) Toxin standards. (B) Clam extract containing $24 \mu\text{g}/100 \text{ g}$ PSP. (C) Mussel extract (diluted 1:2) containing $388 \mu\text{g}/100 \text{ g}$. (Reproduced from ref. 115 with permission from AOAC International, Arlington, VA.)

raphy, where high efficiency silica-based reverse-phase columns were employed and rather than using gradient elution, three isocratic ion-pair mobile phases were developed to determine separately the "C" toxins, the GTX toxins, and neosaxitoxin and saxitoxin. In addition, the modified system is capable of separating and quantitating several decarbamoyl compounds (e.g. dc-saxitoxin, dc-GTX2 and dc-GTX3) which is not possible with the gradient system employing the polymer based PRP-1 column. Also, the detection limits for individual toxins were significantly improved because of the higher efficiency separations, compared to results obtained with the PRP-1 column. Figure 8.16 shows typical separations obtained with this system.

8.3.1.2. Prechromatographic oxidation reactions

Because of the time and special equipment required for setting up the postcolumn oxidation apparatus, it is not particularly well suited for laboratories in which PSP analyses are only a part of their work and not carried out on a regular basis. As a result, attempts have been made to develop a simplified HPLC method employing prechromatographic oxidation to form fluorescent derivatives of the individual toxins. In this approach, the oxidation reaction is carried out before the chromatography and the oxidation products are separated by HPLC and then quantitated directly with no postcolumn equipment. The oxidation procedure is particularly attractive as a means of derivatization since the reaction is simple, requiring only dilute periodate or peroxide at weakly basic pH. Also, the reagents themselves are non-fluorescent and thus do not interfere in the detection of the products. Luckas [121] first reported on the HPLC analysis of oxidation products of saxitoxin after H_2O_2 oxidation. He observed a number of products and concluded that precolumn oxidation followed by HPLC would not be suitable as a reliable method for PSP determination. However, Preun et al. [122] studied the reaction and improved the chromatography so that saxitoxin produced one major peak with alkaline peroxide oxidation. This method was applied to the determination of saxitoxin in mussel samples.

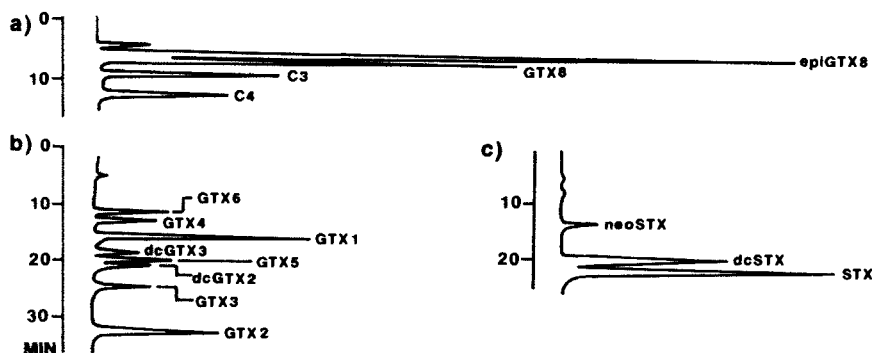


Fig. 8.16. Chromatograms of standard PSP toxins obtained using mobile phases containing (a) 1 mM tetrabutylammonium phosphate pH 5.0, (b) 2 mM sodium heptanesulfonate in 10 mM ammonium phosphate (pH 7.2) and (c) same as (b) but with 10% acetonitrile. epi GTX8 = C1, GTX8 = C2, GTX6 = B2, GTX5 = B1. (Reproduced from ref. 120 with permission of Elsevier, Amsterdam.)

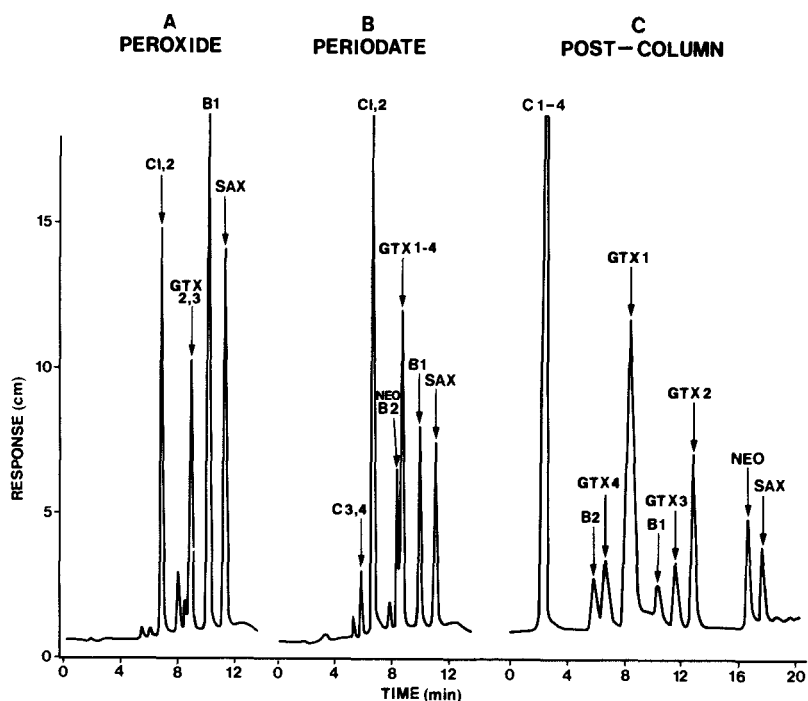


Fig. 8.17. Chromatograms of a PSP toxin standard solution. Prechromatographic oxidation (A) with peroxide and (B) with periodate. (C) Postcolumn oxidation with periodate. Quantities of saxitoxin injected (A) 0.6 ng, (B) 0.5 ng, (C) 3.7 ng. (From ref. 123 with permission of AOAC International, Arlington, VA).

The prechromatographic oxidation method was studied extensively by Lawrence and co-workers [123,124] who evaluated both peroxide and periodate for the oxidation of PSP toxins under a variety of reaction conditions. They reported that the non-N-1-hydroxy toxins (saxitoxin, B1, GTX2, GTX3, C1 and C2) produced a single major peak with both peroxide and periodate oxidations. The N-1-hydroxy toxins (neosaxitoxin, B2, GTX1, GTX4, C3 and C4) did not produce fluorescent products with peroxide but did with periodate. However, all N-1-hydroxy analogues produced three fluorescent products with periodate, one of which was predominant for each toxin and could be used for quantitation. The major drawback of the method is that several of the toxins yield the same oxidation products which are not separable by reverse-phase HPLC. However, attempts at improving the quantitative aspects of the method have met with some success since a reasonable correlation with the mouse bioassay was reported [124]. Figure 8.17 shows chromatograms of a standard mixture of toxins determined by both prechromatographic and postcolumn oxidation methods. The biggest advantage of the prechromatographic oxidation method is that it employs only normal HPLC equipment (reverse-phase chromatography with fluorescence detection) and no postcolumn apparatus is required. In addi-

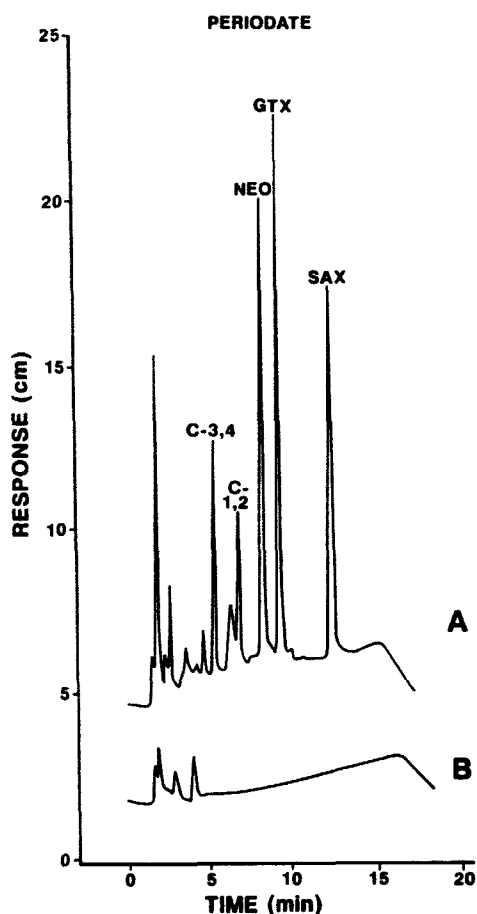


Fig. 8.18. Chromatograms of extracts of (A) contaminated (total PSP equivalent to $100\text{ }\mu\text{g}$ saxitoxin/100 g) and (B) uncontaminated mussels, with periodate oxidation. (Reproduced from ref 124 with permission of AOAC International, Arlington, VA).

tion, the method is significantly more sensitive than the postcolumn method. The detection limits for individual toxins in shellfish tissue are in the low ng/g range making it much more than adequate for monitoring shellfish at regulatory levels (e.g. $80\text{ }\mu\text{g}/100\text{ g}$, total PSP in saxitoxin equivalents). Figure 8.18 shows typical results obtained with extracts of naturally contaminated and uncontaminated mussels. As can be seen, the presence of PSP toxins is clearly indicated in the contaminated sample.

Overall, the prechromatographic oxidation reaction is well suited for monitoring for PSP toxins on an occasional basis where the exact identity of each individual analogue is not required. However, for research purposes where the quantities of each toxin are required, the postcolumn procedure is preferred. Further work on improving the prechromatographic oxidation procedure to separate and quantitate all PSP analogues is in progress in the authors' laboratory.

8.3.1.3. HPLC-mass spectrometry

Several reports have appeared in the literature on the application of MS to the characterization of PSP toxins. FAB ionization MS has provided useful data on a variety of individual PSP analogues [125,126]. However, the technique is not particularly suited to determining the toxins in shellfish samples on a routine basis. The combination of HPLC-MS has been evaluated for identification of PSP toxins in shellfish. Luckas et al [127] evaluated HPLC-TSP MS for the confirmation of decarbamoyl saxitoxin in canned mussels. They used a mobile phase of aqueous ammonium acetate containing acetonitrile to separate decarbamoyl-saxitoxin from saxitoxin. Their analysis was specifically directed at these two toxins. No application to other PSP toxins was presented.

Pleasance et al [128] evaluated HPLC-ion-spray MS for the confirmation of PSP toxins. They found that the chromatographic conditions used to separate the toxins in the postcolumn fluorescence method were not compatible with ion-spray MS. However, they did obtain separation of GTX2, GTX3, neosaxitoxin and saxitoxin using a mobile phase containing ammonium formate and acetonitrile. Figure 8.19 shows an example of the HPLC-ion-spray MS analysis of a dinoflagellate culture containing PSP toxins. Saxitoxin, neosaxitoxin, GTX2, GTX3 and C1/2 were detected and confirmed by selected ion monitoring.

HPLC-MS for phycotoxin confirmation is currently the subject of extensive research by a number of groups. It should lead to some important breakthroughs in the monitoring of PSP and other marine toxins in fish and shellfish.

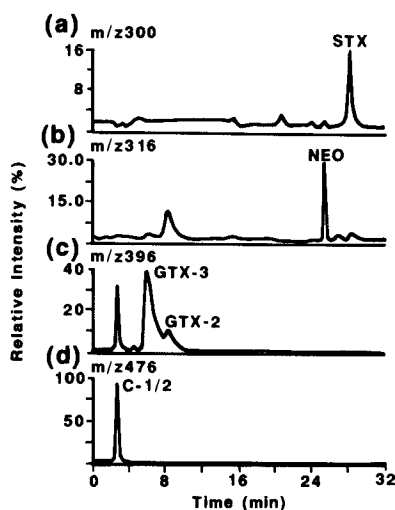


Fig. 8.19. HPLC-ion-spray MS of PSP toxins isolated from dinoflagellate (*Alexandrium excavatum*). Selected ion monitoring of MH^+ ions. Amounts injected, saxitoxin (STX) 908 ng; NEO, 247 ng; GTX2/3 141/396 ng; C1/2, 797 ng. Flow rate 1 ml/min. Split = 20:1, 50 μ l/min entering the mass spectrometer. PRP-1 column with mobile phase described in the text. (Reproduced from ref. 128 with permission of Wiley, New York.)

8.3.1.4. Other techniques

Several other methods for detection of PSP toxins have been reported, some of which are particularly useful in specific applications while others offer potential for the future. Onoue et al [129] detected PSP toxins after HPLC separation by postcolumn fluorescence derivatization with OPA. This reaction, however, appears to be much less sensitive than the postcolumn periodate reaction described above. Also, because the OPA reagent reacts with most primary amines, amino acids and peptides, the method is much less selective, requiring a significantly greater degree of clean-up than the oxidation reactions.

One of the earliest chromatographic techniques for PSP toxins was the thin-layer chromatographic method developed by Buckley et al [130]. PSP toxins are chromatographed on silica gel plates and after development are sprayed with 1% hydrogen peroxide solution and heated. The toxins become oxidized and are visualized under long wavelength UV light or quantitated by scanning. This method has been used successfully in research on isolation and purification of PSP toxins [131].

Electrophoresis [132] and more recently capillary electrophoresis [133] have been successfully employed in the separation and detection of PSP toxins. Although little work has been done on evaluating these techniques for routine screening, they do offer potential.

8.3.2. Diarrhetic shellfish poisons

Diarrhetic shellfish poison (DSP) is a term given to a group of compounds which may be present as contaminants in shellfish as a result of their consumption of certain dinoflagellates. The compounds produce severe gastrointestinal illness in humans, and as a result, there is much concern about their presence in shellfish destined for human consumption. Several countries have established regulatory guidelines limiting the presence of these substances in shellfish. The toxins associated with DSP have been determined to be large polyether compounds as shown in Fig. 8.20. They include the dinophysins, the pectenotoxins and the yessotoxins [134–136]. These toxins, like most of the PSP toxins, are not readily available in sufficient quantity to enable substantial research to be carried out on chemical methods for their detection in shellfish. Because of this, the most commonly used method for DSP determination is one based on a mouse bioassay [137,138].

A number of approaches for the HPLC determination of DSP toxins have been evaluated. These mostly involve derivatization of the carboxylic acid moiety of the compounds to form highly fluorescent derivatives which are then separated by reverse-phase chromatography. The method which has received most attention up to the present is that developed by Lee et al. [139] for the determination of okadaic acid and DTX-1. The method involves extraction of the samples with 80% MeOH in H₂O followed by partitioning with petroleum ether and chloroform. The chloroform extract is then derivatized with 9-anthryldiazomethane (ADAM) to yield highly fluorescent products as shown in Fig. 8.21. After derivatization, the reaction mixture is further cleaned up by passage of the solution through a disposable silica gel SPE cartridge. Figure 8.22 shows typical chromatograms obtained for okadaic acid and DTX-1 added to an extract of mussel digestive glands. As

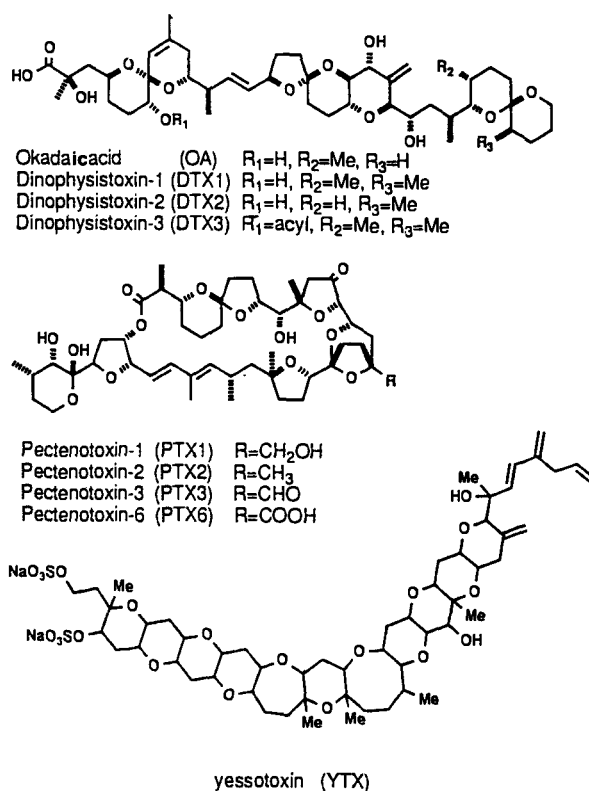


Fig. 8.20. Structures of DSP toxins.

can be seen, both okadaic acid ($3 \mu\text{g/g}$) and DTX-1 ($6 \mu\text{g/g}$) are readily detected at these spiking levels.

A number of modifications of the method of Lee et al. [139] have been reported. An improved clean-up procedure [140] involving a modification of the silica gel clean-up and the use of an internal standard (deoxycholic acid) to monitor the efficiency of the ADAM derivatization reaction enabled the detection of levels as low as 40 ng/g of okadaic acid or DTX-1. A column switching technique which avoided the use of the silica gel clean-up has also been described [141]. In this method, derivatized extracts are injected into the HPLC (reverse-phase) system. The okadaic acid and DTX-1 derivative peaks are “heart-

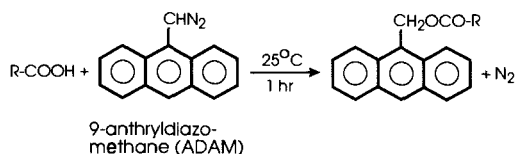


Fig. 8.21. Reaction of 9-anthryldiazomethane with carboxylic acids to form fluorescent derivatives.

cut" and directed onto an enrichment column after which they are switched again to a third column where they are separated and detected fluorometrically. The method appears somewhat complex requiring three columns and switching valves. It has not been evaluated extensively on a routine basis.

Since the ADAM reagent is relatively expensive and of limited stability, a method for synthesizing it immediately before use has been described [142,143]. This approach gives comparable results to that obtained using purified ADAM. Other investigators have evaluated different reagents for forming fluorescent derivatives of okadaic acid and DTX-1. Coumarin derivatives have been studied as suitable replacements for ADAM. The reagent 4-bromomethyl-7-methoxy coumarin was found to yield highly fluorescent derivatives with both okadaic acid and DTX-1 [144]. This reagent is one of a series of coumarin compounds that have been evaluated for forming fluorescent derivatives of carboxylic acids [145]. Several have been compared for application to DSP determination and it was found that 4-bromomethyl-6,7-dimethoxy coumarin produced the most sensitive derivative [146]. Figure 8.23 shows a generalized reaction scheme. Although the coumarin type reagents are stable and reasonably priced, they appear to be somewhat less selective than ADAM, necessitating additional sample clean-up before HPLC determination. However, they do offer potential for routine determinations.

Other reagents which have been evaluated for okadaic acid and DTX-1 are *N*-(9 acridinyl)-bromoacetamide [143,147] and 9-chloromethylantracene [148]. The former in-

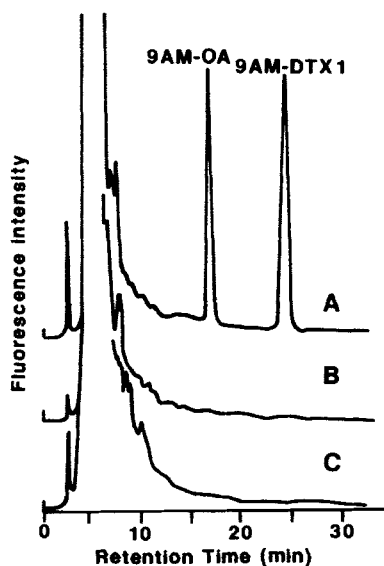


Fig. 8.22. Chromatograms of (A): 9-anthryldiazomethane esters of okadaic acid ($3 \mu\text{g/g}$) (9AM-OA) and DTX-1 ($6 \mu\text{g/g}$) (9AM-DTX1) added to an extract of nontoxic mussel digestive glands. (B): blank nontoxic mussel digestive glands. (C): extract of nontoxic scallop digestive glands. (Reproduced from ref. 139 with permission of the Agric. Chem. Soc. Japan, Tokyo.)

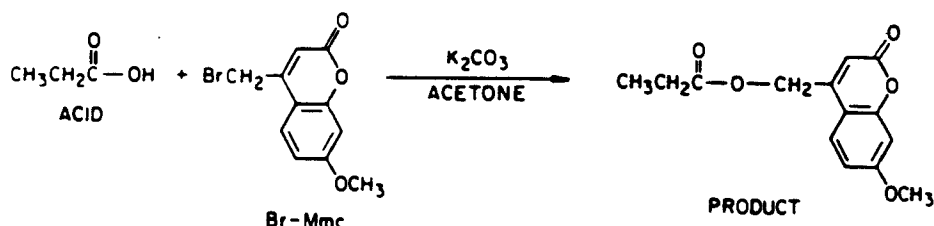


Fig. 8.23. Reaction of 4-bromomethyl-6-methoxycoumarin with carboxylic acids.

involved phase-transfer catalysed esterification in an aqueous/organic two-phase system. As little as 10 fmol of carboxylic acid can be detected with the procedure.

The major problem with all of the above methods is that okadaic acid and DTX-1 are very similar in polarity to many naturally occurring fatty acids. In order for any of these derivatization reactions to work, it is necessary to remove as much as possible potentially interfering carboxylic acids. Further work on the selective clean-up of these toxins should enable their detection limits to be significantly lowered. However, there always remains the trade-off of increased sample clean-up and improved detection limits with analysis time and method reproducibility due to increased sample handling.

The only reports to date on the direct determination of okadaic acid and DTX-1 in shellfish have involved HPLC combined with ion-spray MS [143,149]. With this technique, extracts of shellfish can be analysed directly without derivatization and without clean-up of the extract. Figure 8.24 compares results with those obtained with the HPLC-

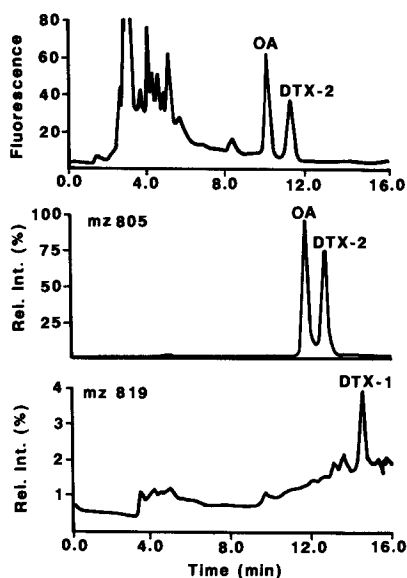


Fig. 8.24. Comparison of HPLC-ADAM (a) with HPLC-ion-spray MS (b and c) for okadaic acid (OA), DTX-1 and DTX-2 in contaminated Irish mussels. (Reproduced from ref. 149 with permission of Wiley, New York.)

ADAM method. A good agreement was found between the two methods. Although the HPLC-MS equipment is too expensive for most routine analytical labs, this work shows that the approach offers excellent potential for confirming positive findings by the HPLC-ADAM or other technique. It was this HPLC-ion-spray MS method that led to the discovery of a new (DTX-2) toxin in Irish mussels [149]. The detection limits were estimated to be about 10 ng/g for each toxin in digestive glands of mussels.

Very little analytical method development work has been reported for the pectenotoxins or the yessotoxins, due primarily to the lack of analytical standards. A fluorometric labelling method for HPLC analysis has been reported for the pectenotoxins [150]. The method involves reaction of the toxins with 1-anthroyl nitrile which reacts with primary hydroxyl groups [151]. It has been applied to the determination of PTX-1 and PTX-4 in scallops and to PTX-2 in *Dinophysis fortii* with FAB MS confirmation.

The same authors [150] reported on a direct HPLC (reverse-phase) method for the detection of YTX and 45-hydroxy-YTX in Norwegian and Japanese mussels. The method made use of the natural UV absorption of the compounds at 230 nm. More work needs to be carried out on these methods to determine accuracy, reproducibility and detection limits which are required before they can be employed for regulatory purposes.

8.3.3. Domoic acid

Although domoic acid (structure, Fig. 8.25) was first isolated in 1958 from the red alga *Chondria armata* [152], it was not until an outbreak of domoic acid poisoning in humans in 1987 [153] that research into method development was carried out to any great extent. The first analytical method for determining domoic acid in shellfish involved reverse-phase HPLC analysis with UV detection of the underivatized compound at its absorption maximum of 242 nm [154]. Since then several other methods have been reported, differing mainly in extraction procedure [155–157]. These methods either employed boiling water, boiling 0.1 N HCl or methanol/water as extracting solutions. The last approach appears to be in most common use at present as it does not require heating. The acid extraction procedure is actually the same one that is used for the PSP mouse bioassay. The use of it is convenient if both PSP and domoic acid are to be determined on the same samples. It has been successfully collaboratively studied by AOAC International [158]. However, recoveries were only about 70–75% and the extracts were not stable enough to store for more than a few days. The methanol/water extraction procedure yields cleaner and much

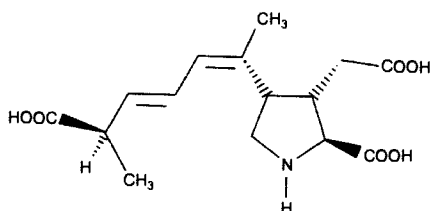


Fig. 8.25. Structure of domoic acid.

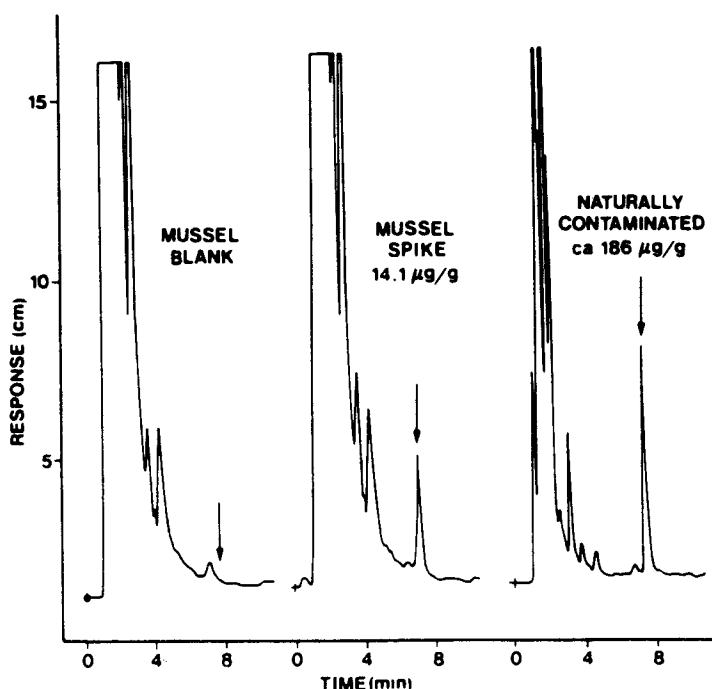


Fig. 8.26. Chromatograms of a blank mussel extract, a blank mussel extract plus 14.1 $\mu\text{g/g}$ domoic acid and a naturally contaminated mussel extract. Arrow indicates domoic acid retention time. (Reproduced from ref. 158 with permission of AOAC International, Arlington, VA.)

more stable extracts. Figure 8.26 shows typical chromatograms of domoic acid in mussel tissue using the collaboratively studied method [158].

Several chemical derivatization methods have been investigated either to confirm results obtained by direct HPLC [159,160] or for detection of extremely low amounts of domoic acid in, for example, sea water [161]. A confirmation procedure need only be as sensitive as the original analytical procedure. Thus the main aim of chemical derivatization in this case is to create a derivative of domoic acid with a different retention time. If, in an unknown sample, a peak is observed at the same retention time and with a similar UV spectrum as domoic acid, then derivatization of that substance should yield the same product as domoic acid if, indeed, the unknown peak was domoic acid. This gives additional information in identifying domoic acid in unknown samples. Several reactions have been evaluated for this. Esterification of the carboxylic acid groups with hydrochloric acid and various alcohols led to the formation of domoic acid triesters [162]. The advantage of this reaction is that the reagents do not contain strongly absorbing chromophores which may yield UV absorbing derivatives with sample co-extractives. However, in spite of this, additional sample clean-up was necessary because sample co-extractives in the shellfish samples interfered by consuming the derivatization reagent, resulting in low esterification yields for domoic acid.

Several N-H reactive reagents have also been evaluated. Phenyl- and butyl-isothiocyanate were evaluated for confirmation using UV detection [159,160]. Phenyl-isothiocyanate, used for amino acid analyses, proved to be somewhat less satisfactory than the butyl reagent since the former produced UV-absorbing derivatives with much of the co-extracted material in the sample extracts that led to interferences in detecting the domoic acid derivative [159]. Additional clean-up of the extracts with SPE cartridges was required. The butyl-isothiocyanate reagent [160] was more successful but it too required additional sample clean-up when the domoic acid levels were in the low $\mu\text{g/g}$ range. Figure 8.27 illustrates the effect of cleaning up a spiked mussel extract on the derivatization of domoic acid. The SPE treatment (strong cation exchange cartridge followed by a reverse-phase C-18 cartridge) provided very good clean-up enabling the confirmation of domoic acid at low $\mu\text{g/g}$ levels.

Only one report has appeared on the fluorescence derivatization of domoic acid [161]. The reagent employed was 9-fluorenylmethyl chloroformate (FMOC). It reacts with the N-H moiety of domoic acid to form a highly fluorescent derivative detectable in sub-nanogram quantities. The method was applied to the detection of 15 pg/ml levels of domoic acid in sea water. This reaction also required additional sample clean-up.

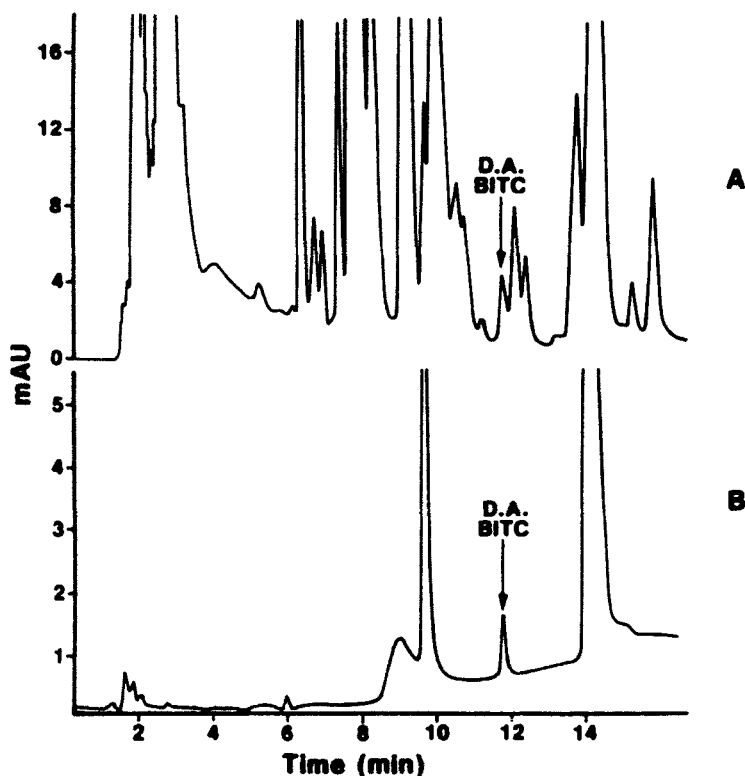


Fig. 8.27. Chromatograms of uncleaned (A) and cleaned (B) extracts of domoic acid (DA) spiked (22 $\mu\text{g/g}$) mussels after butylisothiocyanate (BITC) reaction.

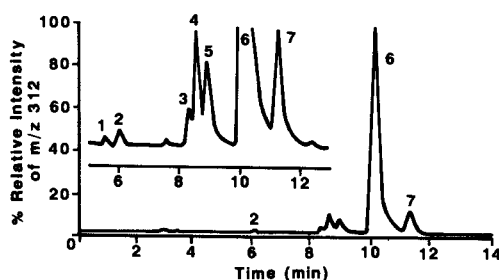


Fig. 8.28. Ion-spray HPLC/MS chromatogram of a toxic mussel extract ($37 \mu\text{g/ml}$ domoic acid, peak 6). Other numbered peaks are domoic acid isomers. Positive ion mode, selected ion monitoring of m/z 312. (Reproduced from ref. 162 with permission of Wiley, New York.)

HPLC combined with ion-spray MS has been shown to be particularly useful for confirmation of domoic acid in shellfish [162]. Figure 8.28 shows a selected ion chromatogram of an extract of domoic acid contaminated mussel tissue (containing $37 \mu\text{g/ml}$ in the extract). As can be seen, domoic acid (peak 6) is readily detected at this level. In addition, all other numbered peaks have been identified as domoic acid isomers. Research on the interrelationship of the isomers, their toxicity and stability is still required.

Capillary electrophoresis, a technique of much interest in analytical chemistry at present, has been investigated for the detection and quantitation of domoic acid [163]. The method employed UV absorbance detection and could detect as low as $3 \mu\text{g/g}$ domoic acid in mussel tissue.

Overall, the HPLC methodology for domoic acid is more than adequate for monitoring at the suggested Canadian guideline level of $20 \mu\text{g/g}$. The direct method requires minimal clean-up and employs HPLC equipment routinely used in most trace analytical laboratories.

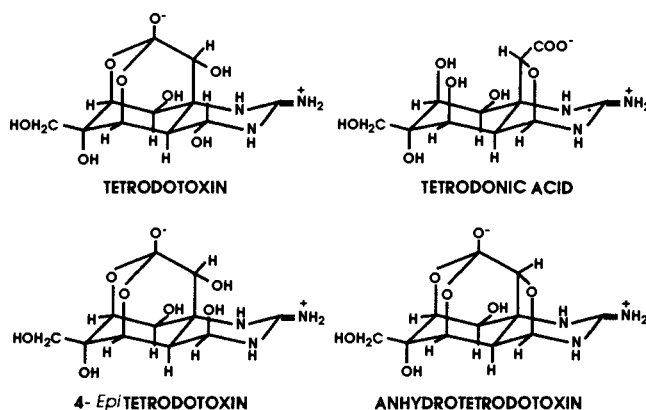


Fig. 8.29. Structures of tetrodotoxin and three derivatives, tetrodonic acid, 4-epitetrodotoxin and anhydrotetrodotoxin.

8.3.4. Other phycotoxins

Tetrodotoxin and its derivatives (Fig. 8.29) are non-proteinaceous toxins found in a variety of fish most notably the pufferfish [164,165]. Nine tetrodotoxin analogues have now been identified [166,167]. Methods for analysis of tetrodotoxin have involved HPLC with postcolumn reaction with NaOH [168–170] or OPA [171]. The latter reaction is much less selective since it gives fluorescent products with any primary amine co-extractives in the sample extract. The postcolumn method employing NaOH yields cleaner chromatograms and could detect low nanogram quantities of tetrodotoxin which enabled the determination of less than 1 µg/g of the toxins in fish tissue.

Other phycotoxins of concern are the brevetoxins (produced by the marine dinoflagellate *Ptychodiscus brevis*), ciguatoxin (from *Gambierdiscus toxicus*) and the freshwater toxins, anatoxins, aphantoxins and microcystins (produced by cyanobacteria). Analytical methods for these compounds are still very much in the developmental stages due mainly to the lack of pure standards for research purposes. HPLC has been used in the purification of brevetoxins [172], ciguatoxins [173] and anatoxins [174].

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Chapter 9

Determination of radionuclides in environmental samples

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9.1. INTRODUCTION

The determination of radionuclides in environmental samples is an important task in relation to the protection of human health. This is especially the case when there is an accidental release of radioactivity into the environment, as was the case with the Chernobyl accident. Assessment of the situation requires knowledge of the type and form of released radionuclides and reliable and practical techniques for the analysis of various radionuclides.

Numerous sources of ionizing radiation can lead to human exposure: natural sources, nuclear explosions, nuclear power generation, the use of radiation for medical, industrial and research purposes, and radiation-emitting consumer products. Before assessing the radiation dose to a population, one requires a precise knowledge of the activity of a number of radionuclides. The basis for the assessment of the dose to the population resulting from a release of radioactivity into the environment, the estimation of its potential clinical health effects and, ultimately, the implementation of countermeasures to protect the population, is the measurement of radioactive contamination in the environment after the release.

The purpose of this review is to summarize the main aspects of the problem of determination of radionuclides in the environment.

9.2. RADIONUCLIDES IN THE ENVIRONMENT

Radionuclides which can be found in the environment can be divided into three groups:

- (1) naturally occurring nuclides of very long half-life which have persisted since the formation of the Earth, and their shorter-lived daughter nuclides;

TABLE 9.1

SOME NATURAL RADIONUCLIDES WITH LONG HALF-LIVES

Radionuclide	Half-life (10^9 years)	Specific activity/ Ci (g of element) ⁻¹	Radio- activity
⁴⁰ K	1.27	8.3×10^{-10}	β
⁵⁰ V	6×10^5	2.8×10^{-14}	β
⁸⁷ Rb	47	2.5×10^{-8}	β
¹¹⁵ In	6×10^5	5×10^{-12}	β
¹³⁸ La	110	2.1×10^{-12}	β
¹⁴² Ce	5×10^6	5.7×10^{-14}	α
¹⁴⁷ Sm	110	3.4×10^{-9}	α
¹⁴⁸ Sm	1.2×10^4	2.2×10^{-11}	α
¹⁴⁹ Sm	4×10^5	8.2×10^{-13}	α
¹⁵² Gd	1.1×10^5	4.1×10^{-12}	α
¹⁷⁴ Hf	4.3×10^6	8.4×10^{-14}	α
¹⁴⁴ Nd	5×10^6	1.2×10^{-13}	α
¹⁹⁰ Pt	700	3.3×10^{-13}	α
¹⁹² Pt	10^6	1.4×10^{-14}	α
²⁰⁴ Pb	1.4×10^8	1.8×10^{-16}	α
²³² Th	14	1.1×10^{-7}	α
²³⁵ U	0.71	1.5×10^{-8}	α
²³⁸ U	4.5	3.3×10^{-7}	α

TABLE 9.2

MASSES OF THE VARIOUS DAUGHTERS IN SECULAR EQUILIBRIUM WITH 1 g OF ^{238}U

Isotope	Mass (g)
^{238}U	1.0
^{234}Th	1.4×10^{-11}
$^{234\text{m}}\text{Pa}$	4.8×10^{-16}
^{234}U	5.4×10^{-6}
^{230}Th	1.8×10^{-5}
^{226}Ra	3.3×10^{-7}
^{222}Rn	2.2×10^{-10}
^{218}Po	1.2×10^{-15}
^{214}Pb	1.0×10^{-14}
^{214}Bi	7.4×10^{-15}
^{212}Pb	4.1×10^{-9}
^{210}Bi	2.7×10^{-12}
^{210}Po	7.4×10^{-11}

- (2) naturally occurring nuclides which have short half-lives on the geological time scale, but which are being continuously produced by cosmic-ray radiation;
- (3) radionuclides released into the environment by man's activity, and by accident.

Some of the long-lived naturally occurring radionuclides are shown in Table 9.1. Some elements in this table result in non-negligible doses to man. For example potassium, which is an essential element, is under close homeostatic control in the body. The average mass concentration for an adult male is about 2 g of potassium per kg of body weight. The isotopic ratio of ^{40}K is 1.18×10^{-1} and the average activity of ^{40}K in the body is about 60 Bq kg^{-1} .

Other nuclides in Table 9.1 which are of particular interest are uranium and thorium isotopes and their series. A number of radionuclides are formed during these decays. For illustration, Table 9.2 shows the masses of the various daughter nuclei in secular equilibrium with 1 g of ^{238}U . Of special interest are the gases, radon (^{222}Rn) and "thoron" (^{220}Rn), which are formed as progeny of uranium and thorium in rocks and soil. They are emitted from the ground into the atmosphere, where they decay and form daughter products, isotopes of polonium, bismuth and lead, which either remain airborne until they decay, or are deposited in rain and by diffusion to the ground.

Radon and thoron and their decay products are the most important sources of radiation exposure to the general public, contributing on average about half of the total effective dose-equivalent received from natural and man-made radioactivity: see Table 9.3. The radon contribution to man's annual effective dose-equivalent from natural radiation sources in areas of normal background is 1.3 mSv, which represents 55% of the total.

The second group includes radioisotopes produced by cosmic rays. The rates of production of radioactive isotopes can be estimated reasonably well from the energy spectra

TABLE 9.3

AVERAGE INDIVIDUAL RADIATION EXPOSURES FROM VARIOUS SOURCES

Radiation sources	Effective dose equivalent per year (mSv)
Natural	
Cosmic rays at sea level	0.37
Radon (^{222}Rn and ^{220}Rn)	1.30
Potassium (^{40}K)	0.30
Other natural sources	0.40
Manmade	
Medical use of radiation	0.4–1.0
Nuclear explosives testing	0.01
Nuclear power production	0.002

TABLE 9.4

HALF-LIVES AND DECAY CHARACTERISTICS OF ATMOSPHERIC COSMIC-RAY PRODUCED RADIONUCLIDES

Radionuclide	Half-life	Main radiation
^{10}Be	1.6×10^6 years	β 555 keV
^{26}Al	7.2×10^6 years	β^+ 1.17 MeV; γ 1.81 MeV, 511 keV
^{35}Cl	3.00×10^6 years	β 714 keV
^{80}Kr	2.13×10^6 years	K X-ray
^{14}C	5730 years	β 156 keV
^{32}Si	650 years	β 210 keV
^{39}Ar	269 years	β 565 keV
^3H	12.33 years	β 18.6 keV
^{22}Na	2.60 years	β^+ 0.545, 1.82 MeV; γ 1.275 MeV, 511 keV
^{35}S	87.4 days	β 167 keV
^7Be	53.3 days	E.C., γ 477 keV
^{37}Ar	35.0 days	K-X-ray, Bremsstrahlung to 0.81 MeV
^{33}P	25.3 days	β 248 keV
^{32}P	14.28 days	β 1.710 MeV
^{28}Mg	21.0 h	β 0.459, γ 1.35, 0.31, 0.95, 0.40 MeV
^{24}Na	15.02 h	β 1.389 MeV; γ 1.369, 2.754 MeV
^{38}S	2.83 h	β 3.0, γ 1.88 MeV; γ 1.6, 2.17 MeV
^{31}Si	2.62 h	β 1.48 MeV; γ 1.26 MeV
^{18}F	109.8 min	β^+ 0.635 MeV, 511 keV
^{39}Cl	56.2 min	β 1.91 to 3.45 MeV; γ 0.246, 1.27, 1.52 MeV
^{35}Cl	37.29 min	β 4.91 MeV; γ 1.6, 2.17 MeV
^{34}mCl	31.99 min	β^+ 2.48 MeV; e^- 0.142 MeV; γ 1.17, 2.12, 3.30 MeV; 0.511 keV

of primary and secondary cosmic rays and a knowledge of the corresponding nuclear reaction cross sections. A list of radioactive isotopes produced by cosmic rays, their half-lives, and their main radiation is shown in Table 9.4.

The third group of radionuclides comprises those produced by nuclear explosions and those present in the irradiated reactor fuel. It includes several hundred radionuclides, but

TABLE 9.5

FISSION AND ACTIVATION PRODUCTS WHICH MAY BE OF CONCERN IN HUMAN EXPOSURE

Nuclide	Half-life	Fission yield (%)	Major decay
Fission products			
^{89}Sr	50.5 d	4.77	β^-
^{90}Sr , ^{90}Y	28.7 a, 64.1 h	5.76	β^- , β^-
^{95}Zr , ^{95}Nb	61.09 d, 35.0 d	6.51	β^- , β^-
^{99}Mo , $^{99\text{m}}\text{Tc}$	2.747 d, 6.006 h	6.09	β^- , β^-
^{103}Ru , $^{103\text{m}}\text{Rh}$	39.272 d, 56, 116 min	3.03	β^- , β^-
^{106}Ru , ^{106}Rh	372.6 d, 29.92 s	0.4	β^- , β^-
$^{129\text{m}}\text{Te}$	33.6 d	0.661	β^-
^{131}I	8.021 d	2.875	β^-
^{132}Te , ^{131}I	76.856 h, 2.3 h	4.282	β^- , β^-
^{137}Cs , $^{137\text{m}}\text{Ba}$	30.0 a, 2.55 min	6.136	β^- , γ
^{140}Ba , ^{140}La	12.751 d, 1.6779 d	6.134	β^- , β^-
^{144}Ce , ^{144}Pr	284.45 d, 17.28 d	5.443	β^- , β^-
Activation products			
^3H	12.35 a		β^-
^{14}C	5730 a		β^-
^{55}Fe	2.75 a		EC
^{55}Fe	44.53 d		β^-
^{54}Mn	312.5 d		EC, γ
^{60}Co	5.27 a		β^-
^{65}Zn	243.9 d		EC, γ
^{134}Cs	754.2 d		β^-
^{239}Np	2.355 d		β^-
^{241}Pu , ^{241}Am	14.35 a, 432.0 a		β^- , α
^{242}Cm	162.94 d		α
^{238}Pu	87.7 a		α
^{239}Pu	2.411×10^4 a		α
^{240}Pu	6.563×10^3 a		α
^{242}Pu	3.735×10^5 a		α

Note: Half-life is given in minutes (min), hours (h), days (d) and years (a). One year = 365.25 days.

only a limited number contribute significantly to human exposure. These would normally include fission products and activation products. Radioactive noble gases, e.g. ^{85}Kr , ^{133}Xe , are not considered since they are unlikely to contribute significantly to internal exposure via the food chain. Radionuclides produced in fission and activation processes and which may contribute significantly to human exposure in the event of an accident are listed in Table 9.5.

The primary source of radionuclides produced in the fission process and found in the environment is atmospheric testing of nuclear weapons. The public has been exposed to these and other radionuclides for almost five decades, but there has been a substantial decline in atmospheric testing in the past two decades. Therefore the major source of fission product radionuclides in recent years has been from nuclear accidents. A nuclear reactor meltdown could release a spectrum of radionuclides similar to that from a nuclear bomb explosion, but the ratios of nuclides would differ greatly for the two cases. The reason for the differences in these ratios is that during the reactor operation the long-lived

TABLE 9.6

CORE INVENTORY AND ESTIMATE OF TOTAL RELEASE OF RADIONUCLIDES

Radionuclide	Half-life	Inventory ^a (EBq)	Percentage released ^b
^{85}Kr	10.72 a	0.033	~100
^{133}Xe	5.25 d	1.7	~100
^{131}I	8.04 d	1.3	20
^{132}Te	3.26 d	0.32	15
^{137}Cs	30.0 a	0.29	13
^{134}Cs	2.06 a	0.19	10
^{89}Sr	50.5 d	2.0	4
^{90}Sr	29.12 d	0.2	4
^{95}Zr	64.0 d	4.4	3
^{99}Mo	2.75 d	4.8	2
^{103}Ru	39.3 d	4.1	3
^{106}Ru	368 d	2.1	3
^{140}Ba	12.7 d	2.9	6
^{141}Ce	32.5 d	4.4	2
^{144}Ce	284 d	3.2	3
^{239}Np	2.36 d	0.14	3
^{238}Pu	87.74 a	0.001	3
^{239}Pu	24065 a	0.0008	3
^{240}Pu	6537 d	0.001	3
^{241}Pu	14.4 a	0.17	3
^{242}Cm	163 d	0.026	3

^aDecay corrected to 6 May 1986.

^bStated accuracy: $\pm 50\%$, except for noble gases.

radionuclides tend to build up progressively, whereas the short-lived radionuclides tend to reach an equilibrium state at which their rate of decay equals their rate of production. The proportion of various radionuclides produced in the operation of a nuclear reactor changes with the operating time and with fuel burnup.

Radionuclides classified as activation products are created in nuclear reactors and other nuclear devices by the reactions of neutrons with fuel and construction materials. Activation products include the isotopes of the transuranic elements and radioisotopes of hydrogen, carbon, caesium, cobalt, iron, manganese, zinc, and a host of other radionuclides, all of which should be recognized and considered in determining the environmental pathways to human exposure.

As an illustration, Table 9.6 shows a core inventory and an estimate of the total release of radionuclides following the accident in April 1986 at the Chernobyl nuclear power station [1,2]. In addition, there is a list of technologically modified exposures to natural radiation, which includes the following [2]:

- radiation exposures resulting from the coal burning fuel cycle;
- radiation exposures due to geothermal energy production;
- radiation exposures due to exploration of phosphate rock;
- radiation exposure due to the use of different consumer products (smoke detectors, ceramics, glassware, electronic devices, etc.).

9.3. PATHWAYS AND SAMPLES OF INTEREST

In this discussion, we follow ref. 3 and describe samples and pathways relevant to the analysis of radionuclides in foods, and of environmental materials that are part of the immediate pathways leading to contamination of food.

The source of a release and the conditions at the site where it occurs, determine one or more critical pathways in the environment between the point of discharge and man. The season of the year determines to a great extent the magnitude of contamination of different foods or environmental components.

The main purpose of analysis should be fast identification of the most critical samples and the most important radionuclides so that the necessary rapid actions can be carried out. Let us first discuss food items.

Only those foods should be sampled and those radionuclides analyzed whose consumption contributes significantly to population exposure. If, for example, ^{131}I is being released in proximity to cow pastures, its concentration in the milk produced will provide far more meaningful information than its concentration in air, or deposition on forage samples. Nevertheless, measurements of ^{131}I in pasture grass may be very important in providing an indication of the expected concentration in milk. For other circumstances, the need for food sampling should be based on a thorough understanding of agricultural practice and of food consumption in specific areas of interest.

It is recommended that food analyses be based on the determination of radionuclides in individual food items rather than a mixed diet sample. Only the analysis of individual foodstuffs can indicate whether and which countermeasures should be taken to reduce doses. Food sampling for estimation of total consumption should be carried out at the re-

tail level when appropriate; otherwise, it should be carried out at the consumption level. The selection of foods to be sampled can be based on individual diet or food consumption statistics. Analyses of individual foodstuffs should preferably be performed after preparation, taking into account the effect of kitchen activities such as washing, cleaning and cooking.

Milk and milk products are important components of the diet in many countries. Milk is one of the few foods produced over large areas and collected on a daily basis. Its composition is almost identical all over the world, and it is easy to collect a representative sample that can be analyzed in liquid or dried form.

Milk is likely to be contaminated by radioactive iodine and caesium within the first days after a release of volatile radionuclides. Contamination of milk will be greatest when cows are grazing during the fallout period, but even when cows are kept indoors the contamination of milk may occur by inhalation of radionuclides or their ingestion via drinking-water or contaminated feed. Milk from goats and sheep, should be checked periodically over a longer period, because of their grazing habits.

After harvesting, grain and rice are subjected to contamination only during storage, and only the outer layers would be contaminated. If fallout occurs during the growing season, the radionuclides will be transported into the grain and rice through the plant growth process. It is relatively easy to select representative samples of grain and rice at harvest time. If the fallout occurs during the winter, the grain will be contaminated only through root uptake in the next growing season.

Following an accidental release of radiocaesium, meat becomes one of the main sources of dietary contamination. This mainly results during animal grazing, but contaminated drinking-water might also be an important pathway. Inhalation of radiocaesium is not likely to be a significant pathway to meat. Meat sampling should normally be done in such a way that the composite sample is representative of a large number of animals, although screening measurements of individual animals may be necessary after heavy fallout.

Following an accident, contamination of fish in nutrient deficient lakes may constitute a particularly significant pathway to the uptake of radiocaesium by man. Obtaining a representative sample from an area containing many lakes may require some compromise, since the collection of samples from a large number of the lakes may be impracticable. Ocean-fish will not take up as much radiocaesium as freshwater fish because of the dilution through the depth of the ocean and the effective dilution associated with the high potassium content in the water. Particulate-associated radionuclides can, however, be enriched to high levels. Mussels such as *Mytilus edulis*, some species of macro algae, and other filter-feeders quickly take up the contaminants from sea-water and can also be used as biological indicators.

Green leafy vegetables are very prone to external contamination during their growing season. Other vegetables, including root vegetables, may also become contaminated. It is important to obtain representative samples, and the sampling should be planned carefully. In the early stages of fallout, green vegetables can provide a very significant pathway for short-lived radionuclides.

Game, and foods such as mushrooms and berries, can be contaminated markedly, although only in very rare cases would they contribute significantly to the ingestion dose. It

may still be advisable to analyze these foods in order to decide whether the levels comply with international export regulations.

Environmental samples to be analyzed for the activity of different radionuclides include air, water, soil, grass and sediment.

Measurement of airborne radioactivity provides the first opportunity of identifying the spectrum of radionuclides making up the contamination. Radionuclides will appear very rapidly in ground-level air, and air samples can give the first indication of the nature of the contamination. Radioactive materials in the air may result in exposure to man by inhalation, by ingestion of particulate matter deposited on vegetation, or by ingestion of products derived from animals which were exposed to radioactive materials through inhalation or ingestion.

Rain-water and snow are also early indicators of radioactive contamination. In some places drinking-water and rain-water can be significant pathways of short-lived radionuclides such as radioiodine to man or animals. Drinking-water and household-water are potentially important pathways, directly or through their use in food preparation and processing, although dilution, time-delays and water treatment can reduce the contamination levels markedly. Water consumed by livestock and/or used for irrigation purposes can also be a source of radionuclides in foods. Sea-water can be a source of contamination for seafoods such as mussels, shellfish, fish and algae. Water from streams, lakes and ponds should also be considered as a source of contamination.

Contaminated soil serves as a direct source of radionuclides leading to the contamination of all agricultural products. Contaminated soil used in greenhouses could add significantly to the contamination of vegetables.

Grass is a direct pathway of radionuclides to animals and then to man through meat and/or milk. The radionuclide content of grass can provide a basis for deciding whether cattle can be permitted to graze in a given area.

Sediment in all types of water (sea, lake, pond and large or small streams) may be a source of contamination to aquatic organisms. Contaminated sedimentary materials used as fertilizers may also increase the radioactivity levels of soil.

Following a release of radionuclides from a uranium-fuelled reactor to the environment the most important radionuclides to be assessed for internal exposure from the ingestion of food and water, and for the contamination of environmental materials which are parts of the immediate pathways leading to contamination of food, are ^{134}Cs , ^{137}Cs ($^{137\text{m}}\text{Ba}$), ^{131}I and other gamma emitters, the beta emitters ^{89}Sr , ^{90}Sr and tritium, and the alpha emitters ^{238}Pu , $^{239+240}\text{Pu}$, ^{241}Am and ^{242}Cm .

The levels of radionuclides in the environment and food have been extensively compiled by UNSCEAR [2]. In general, the radionuclides of major importance in the contamination of food and environmental samples (materials which are part of the pathways leading to the food) are:

Air	^{131}I , ^{134}Cs , ^{137}Cs
Water	^3H , ^{89}Sr , ^{90}Sr , ^{131}I , ^{134}Cs , ^{137}Cs
Milk	^{89}Sr , ^{90}Sr , ^{131}I , ^{134}Cs , ^{137}Cs
Meat	^{134}Cs , ^{137}Cs ,
Other foods	^{89}Sr , ^{90}Sr , ^{137}Cs ,

Vegetation	^{89}Sr , ^{90}Sr , ^{95}Zr , ^{95}Nb , ^{103}Ru , ^{106}Ru , ^{131}I , ^{134}Cs , ^{137}Cs , ^{141}Ce , ^{144}Ce
Soil	^{90}Sr , ^{134}Cs , ^{137}Cs , ^{238}Pu , $^{239+240}\text{Pu}$, ^{241}Am , ^{242}Cm

This group of radionuclides is most likely to be of concern in terrestrially produced foods. Biological concentration processes in freshwater and marine systems can result in very rapid transfer and enrichment of specific radionuclides. The radionuclides which enter such systems can in certain cases be rapidly accumulated by plankton and algae. These organisms serve as food for higher trophic levels and thus the radionuclides become concentrated in organisms such as oysters, clams and shrimps. Radionuclides of particular concern in freshwater and marine food chains include: ^{54}Mn , ^{55}Fe , ^{59}Fe , ^{60}Co , ^{65}Zn , ^{95}Zr , ^{95}Nb , ^{103}Ru , ^{106}Ru , $^{110\text{m}}\text{Ag}$, ^{125}Sb , ^{131}I , ^{134}Cs , ^{137}Cs , ^{141}Ce , ^{144}Ce and some of the transuranic elements.

Many other radionuclides would be present in debris from a nuclear accident, and their potential contribution to human exposure depends on the type of accident and the circumstances when it occurred. Since there are several types of fuel, the spectra of radionuclides that would be present in accidental releases could be somewhat different. The following four nuclear accident scenarios are considered in details in ref. 3:

- (i) reactor meltdown, with or without failed containment;
- (ii) reactor meltdown with particle containment;
- (ii) nuclear fuel-reprocessing plant release;
- (iv) plutonium fuel-fabrication plant release.

The types and quantities of radionuclides released in each of these scenarios are different. Other nuclear accidents which may result in major atmospheric emissions are:

- plutonium fuelled reactor meltdown;
- breeder reactor meltdown;
- high flux radionuclide production reactor meltdown;
- fast flux reactor meltdown;
- nuclear powered ship/submarine reactor meltdown;
- satellite re-entry and burnup of satellite nuclear power source;
- nuclear weapon destruction by chemical explosion;
- criticality at nuclear materials processing plant;
- fusion reactor fuel loss.

Each of these possible accidents may release a unique spectrum of radionuclides and this should be considered in developing radioanalytical capabilities.

9.4. COLLECTION AND PREPARATION OF SAMPLES

Collection of samples, or sampling, is the method (or procedure) of extracting samples for the purpose of measuring the characteristics which are surveyed. Environmental radiation monitoring is mainly conducted with the aims of estimating an exposure dose for people near nuclear power facilities and of protecting public health and safety. In this case it is necessary to determine monitoring items, emphasizing the processes that result in individual exposure. These are based on the behaviour of radionuclides and on information

about the population distribution, topography and geology, atmospheric phenomena, and for example, the types, quantities and intake of foods.

There are also some general procedures that one should follow. For example, double identities should be placed on samples at collection time. It is advisable to fill in a standard form with all relevant information (date, location, fresh weight, weather, collector's name, etc.).

After collection, the samples must be properly stored to avoid degradation, spoiling, or other decomposition, and to avoid contamination. Proper care must be taken to avoid loss of volatile radionuclides. Short periods of storage before analysis may require refrigeration, or the addition of a preservative such as sodium bisulphite, alcohol or formalin (as in the case of milk) for biological samples. When long periods of storage are needed, it may be preferable to convert the samples to a more stable form immediately after sampling. Drying or ashing of the sample will allow extended storage, but the temperature must be carefully controlled in these operations to avoid loss of radionuclides.

Sample containers must be suitable for storage without degradation especially when acids are added to liquid samples. The absorption of radionuclides from solution is less on polyethylene than on glass. With a few exceptions, almost all sorption losses can be eliminated by the addition of acid, a carrier solution containing stable elements, or a complexing agent.

The sample collection equipment, containers, and sample preparation areas must be kept clean to avoid contamination. Disposable containers should be used whenever possible (plastic bags, aluminium trays, etc.).

Drying reduces the weight and volume of the samples and may also permit a longer storage-time. Samples may be dried in a low-temperature oven at 105°C or at room temperature without significant loss of any radionuclides except iodine. Samples should be dried for a sufficient period of time, at a fixed temperature, to acquire a constant dry weight. Measurements of fresh or wet weight and the dry weight are required. It is important to prevent contamination during the drying procedure. If necessary, freeze-drying may be used to further reduce the loss of volatile radionuclides from the sample. However, this process is very time-consuming and is therefore not highly recommended.

Evaporation is the normal method of concentrating liquid samples. Reasonable care is required when evaporating liquids, particularly milk, with a hotplate in order to avoid spattering and loss of sample. Evaporation lamps usually eliminate the problem of spattering. The evaporation bowl should be made of material that will not absorb the radionuclides. Some radionuclides, such as iodine, tritium and ruthenium, may be lost during the evaporation process. A fast evaporation can be performed satisfactorily using a rotating evaporation system that operates under reduced pressure. Different volumes of the rotating spheres of up to 30 l are available.

Where samples need ashing, low-carbon-nickel trays are adequate for the ashing operations. However, other trays lined with thin-sheet aluminum, which is discarded after each use, may be entirely satisfactory. Trays are easily cleaned with detergents or dilute mineral acids (usually HCl). The temperature for dry-ashing varies but an upper limit of 450°C is recommended. If the sample is not completely dry at the start, an initial drying step at 105°C should be introduced. The ashing time depends on the type and quantity of the material; large samples may require 16–24 h. Dry-ashing should be used only for ra-

dionuclides that do not vaporize at the ashing temperature. Significant loss of caesium will occur above 400°C.

Carrier elements and radioisotope tracers should be added to all sample types before ashing. Measurements of the ashed weight are necessary for calculation of the radionuclide concentrations and yields. We now consider some specific materials in some detail.

9.4.1. Air

Air provides an important pathway through which humans are exposed, by inhalation, to a number of radionuclides. Air also conveys airborne radionuclides that were once sedimented in soil or on plants. Radionuclides that then reach humans through the respiratory system, digestive system, or skin cause both internal and external exposures.

For the analysis of radionuclides in airborne dust, the dust is collected on a filter using a dust sampler. Iodine in the air is collected on an active carbon filter using a dust sampler. Tritium exists in the form of vapour (HTO) or gas (HT) in the air. The HTO is absorbed on silica gel; HT is changed to HTO using a palladium catalyst and then the HTO absorbed on silica gel. Radioactive noble gases such as ^{85}Kr are absorbed on an active-carbon trap cooled with liquid nitrogen.

Several types of filter material are used for collecting aerosol materials (glass, PVC or Microsorban filters). All commercial filter media, when used properly, have adequate efficiencies. The filters are usually compressed to provide a standard counting geometry and are measured by gamma spectrometry, after which they may be dry- or wet-ashed for radiochemical analysis.

Air particulate samplers are usually classified as low-volume air samplers or high-volume air samplers. There are, in addition to these classifications, dust samplers that consist of a combination of a low-volume suction pump and a movable filter-paper system. Characteristics of these samplers are as follows:

- (i) *Low-volume air sampler*: A low-volume air sampler is an apparatus having a suction capacity of up to 20 l/min. It is used for one continuous sampling lasting from several days to 1 week. Filter papers having a diameter of 5 cm and an active-carbon cartridge can be attached as a collecting device.
- (ii) *High-volume air sampler*: A high-volume air sampler is an apparatus whose suction capacity is between 500 l/min and 2000 l/min. It is used for a sample period of 1 day. A filter paper of dimensions 203 mm \times 253 mm (8 in \times 10 in) can be attached as a collecting device.
- (iii) *Dust sampler*: A dust sampler has a suction pump with the same volume as a low-volume air sampler. It is capable of continuous sampling during 1 month when using an attached long filter paper. Most dust samplers used by local self-governing bodies are specially made to have an attached active-carbon cartridge in addition to the long filter paper. They are capable of measuring total beta and alpha radioactivities and the iodine content of the air.

In addition to a proper choice of collecting material (filter paper), a reliable measurement of flow rate is required. Flow meters are classified into rotameters and integrating flow meters. The latter are further classified into wet-gas meters and dry-gas meters. A rotameter has a specially graduated vertical tube, whose diameter increases in the ascending

direction, containing a spinning top-shaped or spherical float. A gas-stream is admitted into the bottom of the tube and the float is held at a vertical position which varies in proportion to the flow rate of gas.

9.4.2. Water

Tap-water should be collected at the water processing (filtration/purification) plants just prior to discharge into the distribution system. If the water is to be collected from a residence, then the pipes should be flushed sufficiently (2 or 3 min) prior to sample collection.

Rain collectors 0.1 m² to 1 m² in area provide adequate collection of rain-water. Automatic sampling devices are commercially available which protect the collector from dry-deposition prior to the rainfall. These samplers start to open the collection area when rain begins to fall and close it when the rain stops. High-walled vessels with smooth surfaces are equally suitable. Some loss of the less-soluble radionuclides will occur on either of these collectors but the loss can be largely recovered (if desired) by washing with dilute acid (0.1N HCl). An alternative method is to filter the water directly through a mixed-bed ion-exchange column, after which the water is drained away. Contamination of rain-water samples by airborne soil and surface dust can be minimized by locating the sampling stations on the roofs of buildings. Overhanging vegetation should be avoided. The most suitable size for the collector depends upon the amount and frequency of precipitation in the area, as well as the frequency of collection.

If water samples have to be stored for any length of time, hydrochloric acid (11 M) should be added to the sample bottles at the rate of 10 ml per litre of sample either prior to sampling or as soon as possible afterwards to avoid absorption of the radionuclides on the walls of the container. The longer the storage time before analysis the more important it is to acidify the water samples.

In addition to the radioactivity analysis of the samples, other information is required, including the:

- (i) atmospheric conditions (weather and surface air temperature);
- (ii) water temperature, pH, salinity and degree of clarity;
- (iii) location (direction and distance from a navigational mark), latitude.

9.4.3. Soil

It is important to identify the radioactive concentrations in soil because it constitutes a path for radioactivity to humans, animals and plants, and is an indicator of radioactive accumulation in the environment. Soil includes submarine sediment and river-bed soil, in a broad sense, but here it includes only soil from uncultivated and cultivated land. The soil to be measured should consist of particles having diameters of 2 mm or less.

Sampling locations should not have obstacles nearby (trees, structures) and the utilization of the land should be considered. Also, sampling locations should not have unusual soil quality or topography, and should have little vegetation. Locations should be selected for periodic sampling to be possible, in order to determine the accumulation of radioactivity. Samples of earth transported from another place should be avoided, even when the

soil has been mixed. Maps of the sampling locations should be sketched, or photographed whenever possible. Samples should be collected from the surface layer 0 to 5 cm deep, using at five to eight locations a soil sampler having a diameter of 10 cm.

Submarine sediment is an important material for understanding the accumulation of radionuclides discharged with waste-water from nuclear power facilities. Grains analyzed should have a diameter of 2 mm or less.

Samples should be collected at the outlet of a facility drainage duct. Also, supplementary survey samples should be collected offshore. One should refer to marine charts or consult fishermen who are familiar with the region, because sampling may sometimes be hindered by a bedrock, even though the sampling location may have been selected with consideration of the ocean currents.

9.4.4. Biota

Plants take in radionuclides which have been discharged into the environment. In turn, people eat these plants or take them in through animals that have directly or indirectly eaten the plants. It is therefore important to measure the concentration of radioactivity in plants and animals when evaluating exposure doses of humans.

Measurements should be made on milk, a major food for infants and a daily food for many people, to directly estimate the internal exposure dose.

Measurements should also be made on indicator plants and animals, which are neither edible nor directly involved in the human food chain. These indicators grow readily, concentrating radionuclides and when near nuclear power facilities are very useful for monitoring changes in the level of environmental radioactivity.

Next we describe some of the objectives used in sample pretreatment. The objective of these procedures is to reduce the volume of the samples. Portioning, evaporation, concentration, chemical separation, absorption, and so forth, are techniques which can be used alone or in appropriate combinations for liquid samples. Drying, sieving, pulverization, mixing, reduction, ashing, and so forth, are used alone, or in an appropriate combination, for preparing samples of solids for measurement.

The sample pretreatment procedures used will depend on the type of samples and of activity to be measured. Let us mention some of the pretreatment procedures for γ -ray spectroscopy. As examples, Figs. 9.1 and 9.2 show the sequence of steps to be taken during preparation of soil samples and agricultural product samples for gamma spectrometry.

9.5. COUNTING

For the measurement of the concentrations of radionuclides in environmental samples, and especially for low concentration levels, it is essential to reduce the background rate as well as improving the counting efficiency.

In our consideration of the background rate reduction, it is important to pay attention to the origins of the background. These are:

- (1) natural and artificial radioactivity in the environmental circumstances;
- (2) radioactivity in the detector and/or shielding material;

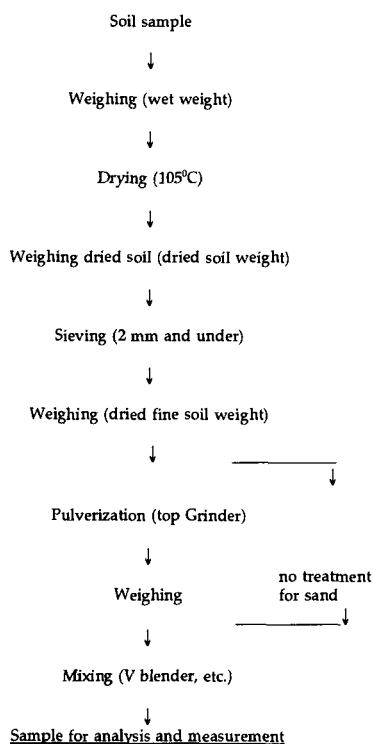


Fig. 1. Pretreatment of soil samples.

- (3) cosmic radiation;
- (4) Instrumental noise.

In the surrounding environmental materials, for example a concrete wall of a building, the soil, air or water, there are various kinds of natural and artificial radionuclides which give rise to a background. The most important are ^{40}K , ^{226}Ra and its decay products, and ^{232}Th and its decay products. As a result, many lines originating from these nuclides are observed even in a heavy shielding box, during the spectrometry with a Ge detector. The background due to these radiations can be reduced considerably by shielding with heavy materials such as lead and iron. Typically, such background can be reduced to a hundredth by a 10 cm thickness of lead or 30 cm of iron. In making a shielding box, it is important that the detector is surrounded entirely (4π direction) with shielding. In choosing the shielding material, one has to pay attention to any undesirable contamination with radioactive substances. For example, a small amount of $^{210}\text{Pb}(\text{RaD})$, which is a member of the ^{226}Ra series, is inevitably contained in lead. Its daughter nuclide $^{210}\text{Bi}(\text{RaE})$ emits energetic β -rays ($E_{\text{max}} = 1.17 \text{ MeV}$), resulting in Bremsstrahlung which may contribute to an increase in background rate. The concentration of ^{210}Pb is very dependent on the mine where it was produced, and a careful check on the content of ^{210}Pb is important for achieving ideal shielding characteristics. Since the half-life of ^{210}Pb is 22 years, old lead has much better characteristics. Iron blocks are sometimes used instead of lead. However,

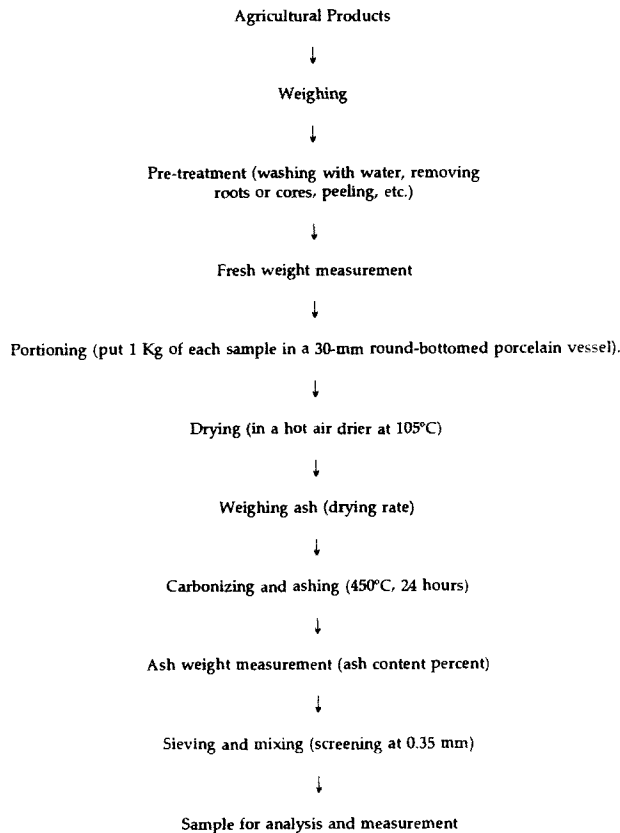


Fig. 9.2. Pretreatment of samples of agricultural products.

the modern iron/steel is often contaminated with ^{60}Co , and careful checking is necessary prior to the construction of the shielding assembly. This ^{60}Co contamination originates from the ^{60}Co source which was used to monitor the abrasion of the furnace wall. For shielding purposes, the use of old iron, for example from materials from a sunken ship, is recommended. The background may also originate from the trace amount of radioactivity in the detector itself and its assemblies, and cannot be eliminated by shielding alone and hence, the contamination of the detector materials with radioactive substances should be carefully examined. A typical example is ^{40}K in the glass used for phototubes. This can be eliminated by the use of a quartz phototube. The ^{226}Ra present in solder sometimes gives rise to a considerable rate of background. The molecular sieve used for keeping a vacuum in the cryostat of a Ge detector sometimes causes a background spectrum. In this sense, electrolytic copper, stainless steel, and perspex are recommended as materials to be used in the shielding box.

Even when very thick materials are used for shielding, it is very difficult to stop the penetration of highly energetic cosmic-rays, which have energies in excess of 10^3 MeV.

In order to overcome this undesirable background, an electrical guard technique is employed.

The cosmic-rays which give a pulse to the main detector should produce another signal to one of the guard counters. The two signals from the main counter and from the guard counter are produced simultaneously. Therefore an anticoincidence technique is applied quite effectively to reduce the background rate, and an ultimate low background rate of the order of 0.1 cpm can be achieved by the combination of heavy shielding and the anti-coincidence guard technique.

The coincidence technique is often used for the low level detection of electrons. As an example, we mention the low background β -ray spectrometer as developed by Tanaka [4]. A small Geiger-Muller counter is mounted inside a large plastic scintillator, and scintillation pulses from only those events which are coincident in the two detectors are analyzed by a multichannel analyzer. In this case, signals caused by cosmic rays are also subject to the analysis, but the minimum width of the scintillator is designed so as to produce a very large pulse-height, and this signal is rejected in the course of the pulse-height analyses. In these instruments, the β -ray spectra can be measured for even very weak samples; for example, the β -rays originating from the ^{40}K contained in the ash of a 3 cm long cigarette. By the addition of a logarithmic amplifier, the analyses of β -ray spectra becomes even easier.

Pulse-shape discrimination is also used sometimes for background reduction. Electrical noise is very often caused by the discharge of high-potential circuits, the transition signal of thyristors and the induction signal from spark-gaps, etc., which result in a background signal to the counter. To prevent this noise, electrical filtering in the main power supply, and electrical and/or magnetic shielding are sometimes required.

Another essential requirement for reliable low level counting is to increase the counting efficiency and/or to use a larger amount of source sample. In order to increase the counting efficiency, one should use a large volume (large size) detector, and try to improve the source-to-detector geometry. However, it should be noted that with increasing detector size or volume, the background may also increase. As for the improvement of the geometry, the use of a well-type detector is advantageous, but the source size is sometimes limited. On the other hand, to use a larger amount of source, it is convenient to use a Malineli-beaker, especially for the measurement of environmental samples such as milk or soil.

In principle one can perform the measurement of activity of any sample by using radiation detection systems and standard sources. Standard sources and solutions are available from many national and international operations. However, for sources one can perform radioactivity measurement without the help of standards.

Absolute counting implies a method of radioactivity measurement performed without the help of a standard/reference source. The methods for the absolute measurements can be classified into two categories. The first comprises the direct methods, which include the defined solid angle method, the 4π counting method, the internal gas counting method, and the liquid scintillation counting method. These methods use relatively simple procedures, and each provides unique applicability. However, some uncertain corrections, such as for the self-absorption, are involved in these methods, and the accuracies obtainable are somewhat limited. The second category is based on the coincidence technique, by

which we can determine the radioactivity more accurately without any uncertain estimation of correction terms.

The most often used methods include: the 2π α -counting method, 4π β -counting with a 4π gas flow counter, 4π β -counting with a liquid scintillation spectrometer, and the 4π β - γ coincidence counting method.

For radioactivity measurements of an α -emitter such as a ^{241}Am uranium source electrodeposited onto a metal disk, a simple 2π α counting with a gas flow proportional counter is practical, and if a reasonable counter is set at the α -plateau region, it responds only to α -particles.

This method is very simple in practice, and can be applied to absolute measurements of very weak sources, without a standard source. With these advantageous features, the method is useful for measurements of samples for environmental radioactivity analysis and also for preparation of laboratory-made standard alpha sources. The classical method of 4π β -counting with a 4π gas flow proportional counter is still useful for the absolute measurement of β -emitting nuclides, provided that good sources with less self-absorption can be prepared. Source activity can be readily calculated from the observed counting rate, after corrections for background, for counting loss due to dead time, and for absorptions.

It is very important to know the extent of absorptions in the source-supporting films and in the source material itself. The self-absorption-values are dependent on both the β -ray energy and the source conditions, and range from one to several tens of percent. In order to obtain reliable data, it is essential to minimize the foil and self-absorptions. The source-preparation techniques are therefore of great importance. As materials for the source support, a VYNS film (copolymer of vinyl chloride and vinyl acetate) coated with thin gold layers is often used. Such thin VYNS films, whose thickness ranges from about $10\text{ }\mu\text{g}/\text{cm}^2$ to $30\text{ }\mu\text{g}/\text{cm}^2$, can easily be prepared.

The sources are then prepared by simple deposition of a known amount of radioactive solution of the sample onto the support. The solid content of the sample solution must be kept to a minimum to minimize the self-absorption. Even when the carrier concentration is small ($0.01\text{--}0.05\text{ mg}/\text{cm}^2$), considerable self-absorption is unavoidable. This is mainly due to the local agglomeration or crystallization formed in the dry state.

Although the attainable accuracy is limited to around a few percent in the simple 4π β -counting method, the method can be applied to the measurement of weak sources, and hence can play a special role in environmental radioactivity measurements after chemical separation.

A liquid scintillation counter is considered to be a version of a 4π counter, since the radioactive material is mixed in a scintillator, and the effective solid angle sustained from the source to the detector is nearly 4π . A larger amount of sample solution can be used in liquid scintillation counting than the samples used for the ordinal 4π counter and this is one of the advantages of liquid scintillation counting. Although the pulse-height may be reduced with distortion due to the quenching phenomena, so that the counting efficiency changes with the source conditions, the possible loss from the quenching effect can be corrected by using pulse-height information. With this method the majority of pure β - and β - γ emitting nuclides, including ^3H and ^{14}C , can be measured accurately and readily by the use of only one or two common standard samples, without the need to prepare

quenching correction curves. In the relationship between the efficiencies for the standard sample and the count-rate of the sample to be measured, a counting efficiency of 100% may be assumed for the standard sample to allow the radioactivity of the sample to be measured.

Alpha counting in the 4π geometry, using liquid scintillation spectrometers, allows a method for the α -radioactivity measurements of environmental samples.

Among various techniques used for radioactivity standardization the 4π β - γ coincidence counting method is undoubtedly the most important technique: most radionuclides can be standardized using this technique or its extensions. Most national standardizing laboratories in the world employ this method for establishing the national standards of various kinds of radionuclides.

The 4π β - γ coincidence counting is an improved version of the β - γ coincidence counting method. A 4π gas flow type proportional counter is usually used as the β -detector, and a NaI(Tl) scintillation counter located near the 4π β counter wall is employed to detect the γ -rays.

The 4π β - γ coincidence counting technique can be readily applied to absolute measurements of electron capture nuclides such as ^{51}Cr , ^{54}Mn , ^{68}Y , or ^{78}Se , since a 4π gas-flow counter flowed with P-10 gas (a mixture of Ar + 10% methane) has a considerable detection efficiency for the electron capture events via detection of the characteristic X-rays and Auger electrons. This should be called 4π X- γ coincidence counting, but the measuring procedures are essentially those for the 4π β coincidence technique.

The 4π β - γ coincidence counting method and its extended techniques are very useful for the precise absolute measurement of radioactivity, which helps to explain why they are employed for the establishment of national standards of radioactivities of various nuclides in most national standardizing laboratories. Laboratory-made standard sources can be prepared by these techniques. However, the techniques are not adequate for measurements of low level radioactivities, and so their direct application to measurements of environmental samples is difficult.

9.6. GAMMA SPECTROMETRY

High-purity detection systems having a very low background are suitable tools for the direct measurement of low-level radioactivity in environmental samples. The background features of the detection system are of considerable importance because they have to be known for one to obtain an estimate of the detection limit and of the minimum detectable activity [5]. The natural radioactivity background originates from the uranium and the thorium series from ^{40}K and from cosmic rays. Natural radioactivity is found in most materials, and it is necessary to shield the detector using carefully selected materials of high density. The materials used in the detector assembly, and the shielding materials need to have the lowest possible inherent background radiation. Containers such as Marinelli beakers can be filled with aliquots of environmental samples and placed on top of the end cap of the detector in an accurately reproducible beaker-detector geometry. The radioactivity of the samples can be measured if the detector has been calibrated with Marinelli-beaker standard sources (MBSS) of the same dimensions, density and chemical composi-

tion. The calibration procedure should follow as closely as possible that defined in the IEC Standard 697/1981 [6]. This method enables the simultaneous detection of several gamma emitters present in the sample matrix without the need for separation of the radionuclides from the matrix.

The method can be applied to a large variety of environmental and biological materials such as air, water, soil, sediments, vegetation (grass, hay, etc.) and, particularly, individual foods of vegetable and animal origin as well as total diet mixtures. This method is suitable for the surveillance and monitoring of radioactivity originating from the operation of nuclear plants, nuclear weapons tests, and releases from nuclear accidents [7].

It is recommended [3] that the gamma spectrometry system shall be a fully integrated data acquisition and computation system comprising the following items:

- A vertical, high purity germanium (HPGe) or a lithium-drifted germanium Ge(Li) detector is recommended. The detector should have an efficiency of 18–20%. Generally, the efficiency of germanium detectors is specified as the photo peak efficiency relative to that of a standard 7.62 cm × 7.62 cm cylindrical NaI(Tl) scintillation crystal and is normally based on the measurement of the 1.33 MeV γ -ray photo-peak of a ^{60}Co source with a source-detector spacing of 25 cm used in both measurement systems. The resolution of the detector which is normally specified for germanium detectors as the full width (in keV) at half maximum (FWHM) of the full energy peak of the 1.33 MeV peak of ^{60}Co should be between 1.8 keV and 2.2 keV. It is recommended that the peak-to-Compton ratio of the detector be greater than 46:1. The peak-to-Compton ratio is defined as the ratio of the count in the highest photo-peak channel to the count in a typical channel just below the associated Compton edge and is conventionally quoted for the 1.33 MeV γ -ray photo-peak of ^{60}Co .
- A preamplifier is necessary. This normally an integral part of the detector unit and is located very near the detector in order to take advantage of the cooling which is necessary for the operation of the detector and which helps the preamplifier to operate with low noise.
- A biased high voltage power supply is required to supply high voltage to the detector through the preamplifier. A power supply of 1500–5000 V is adequate for the operation of germanium detectors.
- A linear amplifier is required to process the output signals from the preamplifier.
- A detector shield will be needed with a cavity which is able to accommodate large (up to 4 l) samples, constructed of either lead or steel with some type of graded liner to degrade X-rays. Lead shields have a much lower back-scatter effect than steel shields. Typically, lead shields have walls 5–10 cm thick, lined inside with graded absorbers made of cadmium (~1.6 mm) and copper (~0.4 mm). Other materials, such as plexiglass and aluminium, are also used as absorbers.
- A multichannel analyzer (MCA) with a minimum of 4096 channels should be connected to a keyboard and display screen for input and output of data and interaction with a computer. Several kits are available for the conversion of personal computers (PCs) into MCAs. Basically there are three types of conversion kits. One makes use of a board with an analogue-to-digital converter (ADC) that simply clips into the PC; a second type uses a clip-in board with an external ADC; and the third type uses a multichannel buffer (MCB) connected to the PC. All of these PC-based MCA sys-

tems are relatively inexpensive and are very suitable for use in germanium and sodium iodide γ -ray spectrometry.

- A rapid data-storage and recovery system is needed. It can consist of magnetic tape, hard disk, floppy disk, or a combination of these media. This system can be used for programming, short-term storage of data, and archiving data.
- A high-speed printer is required for data output. Useful, but not absolutely necessary, is a plotter for archiving spectral drawings.
- Software for system operation and data reduction is usually supplied with the MCA system. Software packages with varying features and capabilities are available for MCAs based on PCs.

Several aspects of gamma spectrometry with such a system deserve some discussion. Interferences associated with gamma determinations may be caused by improper spectral identities, changes in background, errors in calibration and/or geometry, and lack of homogeneity in samples. Many of the problems in γ -ray spectrometry are due to malfunctions of electronic components. Very important also is the calibration of the measuring systems; both energy and efficiency calibration should be performed with care.

Energy calibration of a germanium detector system (i.e. establishing the channel-number of the MCA in relation to a γ -ray energy) is achieved by measuring mixed standard sources of known radionuclides having well-defined energies within the energy range of interest, usually 60 keV to 2000 keV. The use of the lower energy photons emitted by ^{241}Am may indicate changes in the intercept. Mixed γ -ray standards are available in various forms and containers from reliable suppliers. A partial list of radionuclides usually available with gamma energies in the range of interest includes: ^{241}Am , ^{109}Cd and ^{57}Co , ^{139}Ce , ^{51}Cr , ^{22}Na , ^{54}Mn , and ^{60}Co . The energy calibration source should contain a selection of radionuclides with at least four different γ -ray energies. It is recommended that one of the nuclides should be ^{137}Cs . The gain of the system should be adjusted so as to position the 662 keV photo-peak of ^{137}Cs at about one-third full scale. It is also recommended that the gain of the system be adjusted to 0.5 keV/channel. Once these adjustments are made, the gain of the system should remain fixed.

An accurate calibration of the efficiency of the system is necessary to quantify the radionuclides present in a sample. It is essential that this calibration be performed with great care because the accuracy of all quantitative results will depend on it. It is also essential that all system settings and adjustments be made prior to determining the efficiencies and should be maintained until new calibration is undertaken. Small changes in the settings of the system components may have slight but direct effects on the counting efficiency.

Most laboratories involved in radiation measurements now use personal computers and commercially available software for the analysis of γ -ray spectra. Some of these programs allow the user to control the multichannel analyzer (MCA), calibrate the detector for various geometries, and provide analysis results. The programs are easy to use and do not require the user to be an expert in γ -ray spectrometry. Sanderson [8] evaluated of commercially available IBM PC-compatible software in 1987. At that time, it was reported that most of the programs satisfactorily detected peaks and resolved doublets of equal intensity. Problems arose when the doublets were of unequal intensity or the analysis of a complex spectrum was needed. The suppliers of the programs involved in that study have corrected some of these deficiencies. Since many of these programs have undergone numer-

ous revisions, and a few new programs have become available, a re-evaluation was performed [9].

Six programs were evaluated in this study: GAMMA-W (Gesellschaft für Kernspektrometrie, Germany), INTERGAMMA (Intertechnique Instrumentation Nucleaire, France), QSA/Plus (Aptec Nuclear, Inc, Canada), OMNIGAM (EG&G Ortec, USA), GDR (Quantum Technology, Inc., USA), SAMPO 90 (Canberra Nuclear Products Group).

Hardware requirements were similar for all the programs tested (IBM PC compatibility, 384–640K of memory, a hard disk drive). Two programs, QSA/Plus and SAMPO 90, also required a maths co-processor. The QSA/Plus program had to be installed in Windows 3.0. All of the other programs operated directly from DOS. Except for GAMMA-W, all the programs control data acquisition. GAMMA-W, which is written by an independent company that does not manufacture nuclear instrumentation, does not allow the user to read ten different formats of γ -ray spectra. The conclusions reached by the authors were as follow:

All of the programs satisfactorily found small peaks using sensitivity values recommended in the program manuals. However, these values may not be optimal for every situation. When the sensitivity values were lowered, additional valid peaks were found. When the recommended sensitivity values were used, only two programs did not report any false peaks. All of the programs were able to resolve equal-intensity doublet peaks with only a 2 keV (4-channel) separation. The resolution of doublets of unequal intensity, especially where the smaller is on the high-energy side of the predominant one, has improved since the last evaluation. However, some programs still require improvement in this area.

9.7. BETA PARTICLE SPECTROMETRY

Table 9.7 lists beta emitting radionuclides; since beta emitters show a continuous emission spectrum, the average energy E_B and the maximum energy E_B^m are given. Measurement uncertainties are shown in brackets after the respective value (in units of the last significant digit).

The classical way to measure low-level beta particle activity with a Geiger-Müller (GM) gas-flow counter, in anticoincidence with a guard detector, gives a fairly low background (0.003 s^{-1}) and a counting efficiency of 40%. The disadvantage, however, is its inability to give energy resolution. It has been shown [10] that ion implanted detectors can be used not only for alpha particle spectrometry but also for beta particle spectrometry. Their drawback, however, is the high background around 100 keV and noise below 1 keV.

In a recent publication, Olsson et al. [11] have described a detector system for low-level beta particle spectrometry where the good characteristics of gas flow and silicon detectors are used. The gas-flow GM counter used in the detector system is a windowless single-channel version of the GM-25-5 multicounter developed in the Riso National Laboratory, Denmark. The gas-flow counter utilizes a GM counter with a diameter of 22 mm and a guard counter of $80 \times 90 \times 10 \text{ mm}$, using argon (99%)/isobutane (1%) as counting gas. Background counts, produced in the sample counter by cosmic radiation,

TABLE 9.7

SOME COMMON BETA EMITTERS

Nuclide	Half-life	E_β^m	E_β (%)	E_γ
^3H	12.35(1) a	18.6	5.68	
^{14}C	5730(40) a	156.48	49.47	
^{32}P	14.29(2) d	1710.4	695.0	
^{35}S	87.44(7) d	167.47	48.80	
^{55}Fe	2.75(2) a			K_α 5.9 K_β 6.5
^{63}Ni	96(4) a	65.87	17.13	
^{89}Sr	50.5(1) d	1492	583.1	
^{90}Sr	28.7(3) a	546.0	195.8	
^{90}Y	64.1(1) h	2284	934.8	
^{125}I	59.3(2) d			K_α 27.4
			K_β 31.0	
^{210}Pb	22.3(2) a	16.5	4.15 (80)	
		63.0	16.13 (20)	
E total:		6.51		

are reduced by means of the guard counter and attached anticoincidence circuits. As the energy-discriminating detector a passivated implanted silicon detector, with an active area of 450 mm² and a depth of 300 μm , was used. The PIPS detector has a 0.5- μm -thick aluminium coating on the front surface. The detector is placed on top of the gas-flow counter, and integrated into the gas detector unit, allowing the aluminized front surface to act as one of the ground-electrodes of the sample counter. The source is placed in a cavity inside the detector, between the sample and guard counter [11]. The instrumentation used for PIPS detectors is identical to that used for alpha particle spectrometry.

The authors have demonstrated that it is possible, by the coincidence technique, to reduce the contribution of noise and background from a PIPS detector by a factor of ten and to improve its energy resolution. Such a detector system could be a useful tool for quality control of low-level low-energy pure beta emitters such as ^{63}Ni from environmental samples.

9.8. ALPHA PARTICLE SPECTROMETRY

Common radioisotope sources of alpha particles are listed in Table 9.8. All but the first one listed are members of radioactive decay chains. Some of the alpha emitters may decay to several discrete energy levels of their daughters; in this case the branching ratio is indicated.

The detection of alpha particles is based on the physics of the processes which take place when the particles pass through the matter. During this passage the alpha particle

TABLE 9.8

COMMON ALPHA EMITTERS

Nuclide	Half-life	Alpha energy (MeV) (% Branching ^a)
¹⁴⁷ Sm	1.07×10^{11} a	2.232(100)
²¹² Bi	60.55 m	6.051(72), 6.090(28)
²¹⁰ Po	138.38 d	5.304(100)
²¹² Po	0.296 μ s	8.784(100)
²²⁰ Rn	55.6 s	6.288(99.93)
²²² Rn	3.821 d	5.490(99.9)
²²⁶ Ra	1600 a	4.602(5.5), 4.784(49.5)
²³² Th	1.405×10^{10} a	3.954(23), 4.013(77)
²³⁴ U	2.45×10^5 a	4.723(27.5), 4.775(72.5)
²³⁵ U	7.038×10^8 a	4.368(12.3), 4.400(57)
²³⁸ U	4.468×10^9 a	4.150(23), 4.197(77)
²³⁹ Pu	2.413×10^4 a	5.105(11.5), 5.144(15.1), 5.157(73.3)
²⁴⁰ Pu	6570 a	5.124(26.4), 5.168(73.5)
²⁴¹ Am	432 a	5.443(13.1), 5.486(85.2)
²⁴⁴ Cm	18.1 a	5.763(23.6), 5.805(76.4)

^aTotal intensity is normalized to 100%.

loses its energy by excitation and ionization of the atoms. The energy loss per unit path length is called stopping power ($-dE/dx$). The stopping power at an energy above 1 MeV is inversely proportional to the alpha particle energy, while for the energy range less than 1 MeV the stopping power is nearly proportional to the energy.

Source thickness has an important effect on the observed alpha particle spectrum. As alpha particles have a relatively large stopping power, the observed spectra for thick sources show some degradations caused by self-absorption. For high resolution spectrometry, thin alpha sources or samples are required. They are usually prepared by electrodeposition or vacuum evaporation. With a source-thickness less than $10 \mu\text{g}/\text{cm}^2$, no effects due to self-absorption are observed. For such thin sources, a Gaussian-shape line spectrum whose width is limited by the detector energy resolution, is observed for a mono-energetic alpha source. When there is an absorber between an alpha source and a detector the observed energy is reduced by the energy loss in the absorber. Absorbers are the entrance window of a detector, the covering material which prevents contamination of a source, air, and so on. In the case of a thin source, prepared by chemical separation, the emitted alpha particles are observed as line spectra by spectrometers having high energy resolution. The intensity of each alpha emitter is easily estimated from the area of the corresponding peak. Even if the peak is super imposed on the tail of another peak, the peak area can be calculated from the shape of the line spectrum.

An exception is found for the plutonium isotopes, ²³⁹Pu and ²⁴⁰Pu. These are used for estimation of a burn up of nuclear fuel. As the energy difference of these alpha emitters is

only 10 keV, the alpha particle spectrum is observed as an overlapped single peak. However, when a Si detector is used, which has an energy resolution of less than 10 keV (FWHM), the overlapped peaks can be analyzed by the least squares fitting technique.

Alpha particle spectrometry is usually performed using Si detectors, which are especially useful for thin and small-area alpha sources. Two types of Si detectors are commonly used; the surface barrier type and the ion-implanted PN-junction type. As the thickness of the entrance window of these detectors is less than $80 \mu\text{g}/\text{cm}^2$ equivalent of Au, the energy loss in these layers is negligible for alpha particle spectrometry.

As an alternative to Si detectors, a Frish grid ionization chamber is sometimes used as a spectrometer of alpha particles. This ionization chamber has a grid between a cathode and anodes, and a sample is put on the cathode electrode. Electrons and ions, which are generated between the grid and the cathode by ionization, drift towards the anode or the cathode, and a signal pulse is obtained. The pulse height obtained from the cathode depends on the emission angle of alpha particles. Only the drift of electrons is observed due to their drift velocity being 1000 times greater than that of ions. However, the height of the anode pulse is proportional to the alpha energy.

The advantage of this counter is that the areas of samples can be made larger than with Si detectors. A commercially available counter of this type has an area larger than 1000 cm^2 . The energy resolution of this counter is 40–50 keV (FWHM) for alpha particles. Several other types of spectrometers can be used in some specific applications. For example, an organic liquid scintillation counter is useful for detection of extremely low-level alpha activities. Alpha emitters, chemically separated from samples, are mixed into the scintillator. The geometrical efficiency is then 100% and a relatively large amount of source can be introduced into the scintillator. The main drawbacks of the counter are its poor energy resolution and its relatively low light output in excitation by alpha particles compared to that by electrons.

Track detectors are also often used for alpha detection. The length of tracks produced by alpha particles is measured with a microscope after appropriate chemical etching. The energy spectrum of alpha particles is obtained from the distribution of their trace lengths. Advantages of this detector are its high sensitivity, good discrimination against β - and γ -ray backgrounds and the acquisition of alpha emitter distribution in samples. Its main drawbacks are a poor energy resolution and the time consuming processing of the microscope reading.

It should be mentioned that the energy of alpha radiation is one of the most important characteristics of radionuclide sources. Knowledge of the alpha particle energy is necessary for the determination of other major characteristics of external radiation of alpha sources, such as the flux and energy flux density, and the absorbed dose rate. Information on the alpha radiation energy is also used for calibrating semiconductor alpha spectrometers. The results of accurate measurements of alpha particle energies play an important role in the evolution of the atomic-mass scale and comprise nearly 60% of the input data for atoms with $A > 200$, and are deciding factors in the design of precision spectrometers for the high-precision energy measurements of alpha particles from radionuclide sources with the minimum attainable uncertainties. A very precise measurement of alpha particle energies can be achieved by the measurement of the alpha particle time-of-flight. Such a spectrometer has been recently described by Frolov [12].

When applied to environmental samples, the procedures schematically presented in Fig. 9.3 should be followed. A special problem is the assay of alpha particle emitters in water samples. There are several procedures in the literature for assaying alpha particle-emitting radionuclides in natural waters. However, few of them are well suited for low-level analysis since they require the handling of large water samples and the application of concentration techniques. Typical water samples do not contain sufficient amounts of some important nuclides for a precise or reasonably rapid measurement. The most common concentration technique is the coprecipitation of the heavy elements by the addition of a carrier. Some of the best-known methods are coprecipitation with iron as the hydroxide, and with calcium and strontium as the oxalates [13]. However, these methods present some disadvantages. These include the transportation of large volumes of water (several, to hundreds of litres) to the laboratory, the isotopic exchange of tracers, the use of large containers and laboratory ware, and the difficulty of recovering the precipitate.

As a consequence of these difficulties, interest in studying in situ concentration techniques with appropriate adsorbents has increased. These methods eliminate the need for preservation, storage, evaporation or coprecipitation of large water samples. One such approach has been described in detail by Crespo et al [14]. According to the authors MnO_2 and Al_2O_3 can be used as adsorbents in the assay of the low levels of alpha emitters in the waters. This technology requires more development work before it can become routine.

Another problem of importance to safeguards and reactor fuel technology is the measurement of the relative abundance of plutonium isotopes. In addition to γ - and X-ray spectrometry [15], alpha particle spectrometry has also been used [16–18]. This usually results in a complex alpha spectrum, with difficulties in obtaining correct amplitudes for overlapping peaks which tail towards lower energies. The ^{239}Pu and ^{240}Pu peaks overlap when

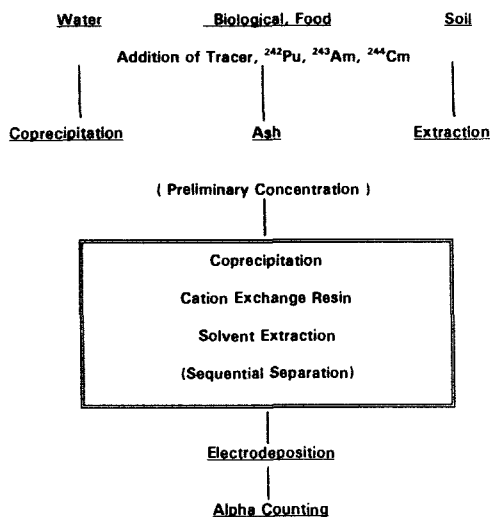


Fig. 9.3.

alpha particle energies are measured using Si detectors. Some sort of deconvolution procedure is required in this case (see, for example, [16]).

9.9. LIQUID SCINTILLATION MEASUREMENT METHOD

Liquid scintillation counting (LSC) has been accepted as the generally preferred method of counting weak beta emitters and is useful to a lesser extent for α - and γ -emitters. The counting sample consists of three components, the radioactive material, an organic solvent or solvent mixture, and one or more organic phosphors. A particle or radiation emitted by the sample material is absorbed in, and its energy transferred to, the solvent and then to the phosphor which emits a scintillation of light photons. These photons are absorbed by the photocathode of a photomultiplier tube which converts them into an electronic pulse. The pulse, after suitable amplification, is registered as a count corresponding to the emission of the particle or radiation.

The technique has the following characteristics.

- (1) Self-absorption is usually negligible.
- (2) There is no absorption of radiation by air or a detector's window between the radioactive sample and the sensitive region of the detector.
- (3) There is no radiation scattering prior to incidence upon the detector.
- (4) 4π counting is performed, because the radioactive material is completely surrounded by the liquid scintillator.

Based on the above merits, the liquid scintillation measurement is extremely sensitive to the low-level radioactivity existing in the environment and food.

Since the radioactive sample material from most methods of sample preparation is in intimate contact or in actual solution with the phosphor, the detection of emitted particles or radiation is highly efficient and may even approach 100%. Problems of self-absorption of the emissions are thus absent, or considerably smaller than those associated with planchette counting of solid samples. This is of particular importance for the measurement of low energy beta emitters such as tritium and carbon-14. On the other hand, the measurement method has intrinsic drawbacks such as quenching and chemiluminescence.

Currently, the liquid scintillation counter has been employed not only for the measurement of low energy β emitters, but also for pure β , β - γ , and α -emitters and further Cherenkov radiation. The liquid scintillator consists mainly of organic solvent and fluorescent material (i.e. solute), and sometimes a surfactant or other material is added to the solution. The characteristics of the liquid scintillator depend mostly on the sort and amount of these chemicals. The liquid scintillator plays the role of an energy transducer, converting radiation energy into photons. The organic solvent which comprises most of the liquid scintillator should satisfy the following conditions[19]:

- (1) The energy should efficiently transfer in the process of luminescence.
- (2) An absorption spectrum of solvent should never overlap an emission spectrum of a solute.
- (3) The radioactive sample and solute must be able to be incorporated with the solvent.
- (4) The solvent must be of high purity.

In the past, many kinds of chemicals were employed as solvents, but nowadays only a few typical solvents are being used including toluene, xylene, pseudocumene and dioxane.

The sample to be measured is easily prepared by incorporating the radioactive sample into a liquid scintillator such as a xylene-base-, toluene-base-, or an emulsive scintillator.

The xylene (or toluene)-base-scintillator is appropriate for hydrophobic samples, to form a homogeneous solution which provides efficient energy transfer and light-counting efficiency.

The simplest mixture consists of a primary scintillator and a secondary scintillator dissolved in a primary solvent. The primary solvent converts the kinetic energy of radiations into excitation energy. The primary solvents are usually aromatic hydrocarbons, and therefore only non-polar radioactive materials can be dissolved in them. The most widely used primary solvent is toluene. Others are *p*-, and *m*-, and mixed xylenes. Pseudocumene (1,2,4-trimethylbenzene) is becoming a popular solvent for new, commercially produced scintillation cocktails. It offers the highest energy conversion efficiency of the solvents known, and has fewer restrictions on shipping and storage as a combustible liquid because of its high flash point, low volatility and lower toxicity [19].

Some of the radionuclides commonly distributed in environmental samples include ^3H , ^{14}C , ^{60}Co , ^{89}Sr and ^{90}Sr . Tritium and ^{14}C emit low-energy betas which are efficiently counted by LSC. Here we present some details of sample preparation for LSC.

Aqueous samples containing tritium are distilled to eliminate impurities and mixed with an emulsifier scintillator with the proportion of 40–50 vol% water. Tritium in biological samples exists as tissue free water tritium (TFWT) and organically bound tritium (OBT). Water containing TFWT is obtained by vacuum distillation (lyophilization) or azeotropic distillation using organic solvents such as toluene and benzene. The OBT is converted into HTO for counting by combusting dried samples.

The tritium concentration in the natural environment is less than 10 Bq/l and sometimes less than 1 Bq/l. Only low-background type LSCs can detect these low tritium levels. To measure tritium below 10 Bq/l with good accuracy, using the conventional type LSCs, it is necessary to enrich the tritium in a large water sample by electrolysis. This procedure can increase the tritium concentration 20–30-fold.

Carbon-14 exists mostly as carbon dioxide (CO_2) and as organic compounds in the environment. Carbon dioxide in the atmosphere or in the effluent gas from a combustion system for biological samples is first absorbed in alkaline solutions such as aqueous NaOH or NH_4OH . If necessary, calcium chloride or barium chloride is added to the alkaline solution to precipitate CaCO_3 or BaCO_3 , which are then purified and stored in a sealed bottle for future analysis. The alkaline solution containing carbon dioxide or carbonate is acidified by titrating with a strong acid solution to generate carbon dioxide. The carbon dioxide gas is then absorbed in a solution of organic amine absorber and liquid scintillator mixture (1:1).

After adding a cobalt salt to sea-water as a carrier, the cobalt-60 is first precipitated as cobalt hydroxide in alkaline solution. The precipitate is separated from the sea-water and dissolved in HCl solution. The ^{60}Co is separated and recovered in the effluent after anion exchange, then evaporated to dryness. The residue is redissolved in dilute HCl solution and transferred into a counting vial. The emulsifier scintillator is added into the vial and mixed well. The counting sample is counted by means of a low background LSC. This

technique can be applied to ^{60}Co in biological samples by using an ashing procedure beforehand.

Strontium isotopes can be measured by utilizing the fact that high energy beta rays in aqueous solution emit Cerenkov photons which can be counted by photomultipliers in a LSC without the need for phosphors.

Strontium radioisotopes in a sea-water sample are separated and purified by precipitation of strontium carbonate and subsequently as a strontium oxalate precipitate followed by cation exchange. Yttrium-90, the daughter nuclide of ^{90}Sr , is scavenged from the solution by $\text{Fe}(\text{OH})_3$ co-precipitation. The acidified aqueous solution containing strontium isotopes is transferred into a counting vial and counted after the total volume of the solution has been adjusted by adding distilled water. By proper setting of the lower level of the discriminator the LSC can count only Cerenkov light signals produced by the 1.49 MeV beta rays of ^{89}Sr , without counting the 0.546 MeV beta rays of ^{90}Sr . Any ^{90}Sr is determined by counting Cerenkov photons generated by the 2.28 MeV beta rays of ^{90}Y which develops in the solution during storing after separation.

9.10. RADIOCHEMICAL ANALYSES

It is not practicable to present here all the methods used; we outline only the reliable methods used for ^{89}Sr and ^{90}Sr , for tritium and for actinides (for details see [3]).

9.10.1. Analysis of strontium

The most commonly used method for separating strontium is by nitrate precipitation. With some modifications this method can be applied to all kinds of environmental samples and foods.

The chemical yield varies according to the type of material. The use of ^{85}Sr tracer to determine chemical yield is a general practice. When determining yield in this manner, it is important that the tracer is pure ^{85}Sr , i.e. free from ^{89}Sr and ^{90}Sr .

Although the method is time-consuming, it is reliable and safe. Rapid methods for ^{90}Sr analysis exist, and it has been shown that they can be used after short-lived nuclides have decayed. In fresh fallout situations, the nitrate precipitation method has been shown to be more reliable. Also, during periods of fresh fallout, the amount of ^{89}Sr is of interest and the rapid methods can only analyze for ^{90}Sr . In the case of higher contamination with ^{90}Sr , the daughter ^{90}Y can be separated without waiting for equilibrium. Within 10 hours the activity concentration of ^{90}Y will be approximately 10% of the equilibrium value and may be sufficient for a reliable ^{90}Sr analysis [3].

A special application of liquid scintillation counters is in the measurement of Cerenkov radiation produce by beta emitters with beta energies greater than 260 keV. This application can be used for screening samples for ^{90}Sr [20–28].

An outline of the method used for determination of radiostrontium in various materials by nitrate precipitation is as follows. The ashed material is dissolved in nitric acid in the presence of strontium and barium carriers. The nitric acid concentration is then increased to precipitate all the strontium and barium (and part of the calcium) as nitrates. After fur-

ther nitric acid separations, barium chromate and iron hydroxide scavenges are carried out. The subsequent treatment depends somewhat on the circumstances but the following is normal practice.

Yttrium carrier is added to the purified strontium solution and, after a delay of about 14 days for the growth of ^{90}Y , the yttrium is separated, mounted and counted. The storage period for the growth of ^{90}Y can be reduced if sufficient ^{90}Sr is known to be present, and the appropriate growth factor applied. For samples of very low activity, as well as for measurement of ^{89}Sr , strontium is precipitated from the solution remaining after the removal of yttrium and mounted for counting. In many cases the determination of the natural inactive strontium content of the material is required so that the strontium chemical yield can be corrected.

In the case of milk, direct application of the nitric acid separation to a solution of the ash usually gives low strontium yields. The calcium strontium and barium are therefore concentrated by an initial phosphate precipitation. The mixed phosphates are then dissolved in an acid and the general procedure continued from that point.

In the case of cereals, and vegetation generally, the ash is very variable in composition and contains numerous elements other than calcium: a mixture of hydrofluoric and perchloric acids is necessary to decompose and dissolve the ash. After heating to remove the hydrofluoric, and most of the perchloric acid, the residue is dissolved in dilute acid and the alkaline earths precipitated as phosphates. For the details of procedure, see ref. 3 and references therein.

9.10.2. Analysis of tritium

Tritium is measured by liquid scintillation counting of a portion of a distilled sample. Several reagents (such as sodium sulphite and silver iodide) can be added in the distillation to prevent interference by radioiodine. The allowed concentration of tritium in water for human consumption is relatively high; thus the method presented here is normally adequate for routine determinations. However, if required, lower concentrations of tritium in water can be determined by electrolytic enrichment. The principles of the tritium determination procedure are as follows.

The water sample is distilled to remove non-volatile quenching materials and non-volatile radioactive materials. Prior to distillation, sodium carbonate (Na_2CO_3) and sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) are added to the sample. The majority of the constituents that might interfere remain in the residue together with any radioactive iodide and bicarbonate that might be present. If the tritium content of non-aqueous biological samples is required, the sample can be converted into water by oxidation. The distillation is carried out to dryness to ensure complete transfer of the tritium to the distillate. An aliquot of the distillate is mixed with a scintillation solution in a counting vial. The mixture is cooled and counted in a liquid scintillation spectrometer (coincidence type). In this sample (usually an emulsion) the kinetic energy of the tritium beta particles is partly converted into light photons. When certain boundary conditions are satisfied (e.g. simultaneous detection by two or more photomultiplier tubes connected in coincidence, discrimination of pulses by preset measurement channels), these photons are counted as pulses.

Standard tritium and background samples are prepared and counted identically to minimize errors produced by aging of the scintillation medium or instrumental drift. The counting rate is a measure of the tritium activity concentration. The sensitivity (counting time 100 min) is generally of the order of 20–200 Bq/l. Details of the method are described in ref. 3. For additional reading see refs. 26, 29–38.

9.10.3. Determination of actinides

Actinides in the environment can be classified into two groups: (i) the uranium and thorium series of radionuclides in the natural environment and (ii) neptunium, plutonium, americium and curium which are formed in a nuclear reactor during the neutron bombardment of uranium through series of neutron capture and radioactive decay reactions. Transuranics thus produced have been spread widely in the atmosphere, geosphere and aquatic environment on the earth, as a result of nuclear bomb tests in the atmosphere, and accidental release from nuclear facilities. Most of these radionuclide inventories have deposited in the northern hemisphere following the tests conducted by the United States and the Soviet Union.

In the actinide series, the elements of greatest interest as environmental contaminants are neptunium, plutonium, americium, and curium, because their presence at relatively high concentrations in ecosystems would represent potential health problems. Nuclear data for actinide isotopes are presented in Table 9.9.

The commonly applied methods for determination of actinides in environmental samples may be classified as follows.

- (1) Preparation
 - (a) drying
 - (b) ashing
 - (c) scavenging
- (2) Solubilization and equilibration
 - (a) fusion
 - (b) leaching
- (3) Concentration and separation
 - (a) coprecipitation
 - (b) ion exchange
 - (c) solvent extraction
- (4) Electrodeposition and alpha spectrometry.

Among ion exchange separation methods for transuranics, strong base anion exchange in hydrochloric and nitric acids are important [39,40]. Among solvent extraction reagents for transuranics, thenoyltrifluoroacetone (TTA) and trioctylamine (TOA) are important [41, 42].

Each transuranic element has many valencies and their behaviour in aqueous solution is very complicated because of disproportionation reactions. As stated above, the ion-exchange and solvent-extraction behaviours of transuranics are dependent on their valency state. Therefore valency control is very important in their analysis [43]. Additional references discussing this problem include refs. [44–56].

TABLE 9.9

NUCLEAR DATA OF ACTINIDE ISOTOPES

Nuclide	α -Energy (MeV)	Yield (%)	Half-life (years)
²⁴⁴ Cm	5.81, 5.76	77, 23	1.81×10
²⁴² Cm	6.11, 6.07	74, 26	6.09×10^6
²⁴³ Am	5.28, 5.23	88, 11	7.37×10^3 tracer
²⁴¹ Am	5.49, 5.44	86, 13	4.32×10^2
²⁴² Pu	4.90, 4.86	74, 26	3.76×10^5 tracer
²⁴⁰ Pu	5.16, 5.12	76, 24	6.57×10^3
²³⁹ Pu	5.16, 5.14, 5.10	73, 15, 12	2.41×10^4
²³⁸ Pu	5.50, 5.46	71, 29	8.77×10
²³⁶ Pu	5.77, 5.72	69, 31	2.85 tracer
²³⁷ Np	4.79, 4.77	51, 36	2.14×10^6
²³⁸ U	4.20, 4.15	77, 23	4.47×10^9
²³⁵ U	4.40, 4.37	57, 18	7.04×10^5
²³⁴ U	4.77, 4.72	72, 28	2.45×10^5
²³² U	5.32, 5.26	69, 31	7.18×10 tracer
²³² Th	4.02, 3.96	77, 23	1.41×10^{10}
²³⁰ Th	4.69, 4.62	76, 23	8.03×10^4
²²⁹ Th	4.90, 4.85	11, 56	7.34×10^3 tracer
²²⁸ Th	5.42, 5.34	73, 27	1.91

9.11. RAPID METHODS

The need for rapid methods is apparent in accident and similar situations. An impulse to the development of rapid methods is also provided through the so-called coordinated research program of IAEA, Vienna. Here we shall present some of the methods reported in a Research coordination meeting on Rapid Methods held in Vienna in 1991.

9.11.1. Radionuclides in bulk food samples

This problem could be approached following the work by Brodzinski and Perkins [57]. They have described a completely portable instrument, operable by one man, which is capable of quantifying the radioactive content of 208-l drums. Eleven radioisotopes are measured simultaneously in just a few minutes. The assayer uses two measuring techniques: segmented γ -ray spectrometry and neutron counting. A drum (or other container) to be assayed is placed on a rotating turntable by a self-contained electric hoist. A collimated high-resolution germanium γ -ray spectrometer vertically scans the rotating drum to measure the intensity of γ rays as a function of the energy emanating from the drum. Most fission and activation products and some transuranic radionuclides emit measurable quantities of monochromatic photons that serve as "fingerprints" of those radioisotopes.

Comparison with the emission rate from known standards provides a quantitative measure of radioactivity from each γ -ray emitter in the drum. This same germanium spectrometer is used to measure the bremsstrahlung radiation from ^{90}Sr . By manipulating the software with the on-board computer, the intensity of the ^{90}Sr bremsstrahlung in the assayed drum is also compared to that of standards, and the ^{90}Sr concentration is quantified. The reported sensitivity for transuranic radionuclides is approximately 1 nCi/g, while that for gamma emitters is of the order of 0.1–1 pCi/g. Also, based on the bremsstrahlung radiation measurement, ^{90}Sr can be determined at concentrations of 100 pCi/g.

9.11.2. Rapid determination of transuranic elements and plutonium

The rapid methods are based on fast removal of the transuranic elements from interfering materials so that they can be electro-deposited as a group, and measured by alpha energy analysis. The procedure involves the following basic steps [58].

- (1) The sample is first brought into solution.
- (2) Radioisotope tracers, including ^{242}Pu , ^{243}Am , and ^{234}Th (if appropriate), are added.
- (3) A small amount of Fe carrier (10 mg) plus sodium sulphite is added to this solution, which is subsequently made basic by addition of ammonium hydroxide to allow the formation of an iron hydroxide precipitate. This precipitate serves to carry the thorium and the transuranic elements.
- (4) The mixture is centrifuged and the solution discarded: the precipitate is dissolved in dilute hydrochloric acid then diluted with water. It is then made basic with ammonium hydroxide, which results in a second iron hydroxide precipitate forming.
- (5) Following centrifuging and discarding of the solution, the precipitate is dissolved in dilute hydrochloric acid, diluted with water, and a small amount of sodium sulphite added to maintain the transuranic elements in their lower valence states.
- (6) A small amounts of calcium and oxalic acid are then added and the pH is adjusted to approximately 3 to allow formation of an oxalate precipitate. The iron forms a very soluble oxalate, thus remaining in solution. This and two subsequent oxalate precipitations serve to remove any remaining iron.
- (7) The final oxalate precipitate is then dissolved in a small amount of sulphuric acid (0.5 ml of concentrated H_2SO_4), and the pH adjusted using dilute ammonium hydroxide.
- (8) The solution is then placed in an electro-deposition cell, where the transuranic elements are electro-deposited on a 1 cm² area of a 2.5 cm diameter stainless steel disc.
- (9) Electro-deposition is conducted for a 1-h period at a current of 1 A.
- (10) Immediately before turning off the current, 1 ml of concentrated NH_4OH is added to the cell and the electro-deposition continued for an additional minute. The current is then turned off, the solution discarded, and the electrode washed with water, then ethanol and air-dried. Following alpha energy analysis, the radiochemical yield, as determined from the radioisotope tracer content and the concentrations of the radionuclides of interest, are calculated.

Samples with a large amount of iron such as soil extracts or vegetation ash may require partial removal of iron prior to initiation of this procedure [58].

9.11.3. Rapid determination of ^{90}Sr

Up to now, two different approaches have been used successfully in fast radiochemical separation procedures for the determination of strontium-90 in environmental sample:

- investigations dealing with the extraction of yttrium-90;
- investigations which tailor precipitation methods for the strontium-90 separation to the needs of special sample types.

We mention only some of these. Vajda et al [59] have described a simple and rapid method for the separation and successive determination of total radiostrontium in soil by using a crown ether. The method consists of three basic steps: oxalate precipitation to remove bulk potassium, chromatographic separation of strontium from most inactive and radioactive interferences utilizing a crown ether, oxalate precipitation of strontium to evaluate the chemical yield. Radiostrontium is then determined by liquid scintillation counting of the dissolved precipitate. When 10-g samples of soil are used the sensitivity of the method is about 10 Bq/kg. The chemical yield is about 80%. The separation and determination of radiostrontium can be carried out in about 8 h.

Another method for ^{90}Sr determination in food and environmental samples has been described in ref. 60. It is based on the use of a tributylphosphate for extraction of ^{90}Y , the daughter of ^{90}Sr . The method is shown to be sensitive to 0.2 Bq per kg of dry grass and milk powder and 2 Bq per kg of soil.

In the case of a nuclear accident, most of the radioisotopes in the environment and food can be reliably and quickly assayed by gamma spectroscopy. There is a problem with some important isotopes which are pure beta or alpha emitters and which cannot be identified directly by gamma spectroscopy. The activity of the isotopes of the strontium group ^{89}Sr , ^{90}Sr and ^{91}Y after a three year reactor fuel cycle can reach about 8% of the total in-core activity, and one of them $^{90}\text{Sr}(\text{Y})$ is important for the long-term health consequences.

It has been shown in ref. 61 that the Ba/Sr reactor core ratio can be used for estimation of the upper limit of the strontium activity in the fallout immediately after an accident. The Cs/Sr ratio can be used for estimation of the strontium fallout in the late postaccident period.

9.12. ANALYTICAL QUALITY CONTROL

Quality control measures are necessary to provide documentation to show that the analytical results are reliable. This is very important since analytical results can form a basis upon which economic, administrative, medical and/or legal decisions are made.

Reliability of results is a function of precision (reproducibility) and accuracy (true value). The precision of results can easily be determined by internal measurement. The determination of accuracy in most cases, however, requires more detailed procedures such as the following:

- Analysis by as many different methods, analysts and techniques as possible. In cases where agreement is good, the results are assumed to be accurate.
- Control analysis with reference materials that are as similar as possible to the mate-

rials to be analysed. Agreement between certified and observed values is then a direct measure of accuracy for that particular determination.

- Participation in an interlaboratory comparison. Samples used in such an inter-comparison should be, as far as possible, similar in composition and concentration to the samples to be analysed on a routine basis. The agreement between the results received from a particular laboratory and the most probable mean value obtained from statistical evaluations of all the results will be a measure of the accuracy for that particular determination.

For practical reasons, most analytical laboratories are not in a position to check accuracy internally, without an external source of reference materials. To overcome some of the difficulties in checking the accuracy of analytical results, the IAEA provides the Analytical Quality Control Services (AQCS) Programme to assist laboratories in assessing the quality of their work. AQCS co-ordinates intercomparison studies and supplies reference materials. Participation is on a voluntary basis and at minimum cost. Information supplied by laboratories taking part in the intercomparisons is treated as confidential.

Reference materials for radioactivity measurements can also be obtained from the following specialized international or national organizations.

- (1) Central Bureau for Nuclear Measurements, commission of the European Communities, Joint Research Centre, Geel (Belgium).
- (2) Office des Rayonnements Ionisants Commissariat a l'Energie Atomique BP 21, 91910, Gif-Sur-Yvette (France)
- (3) Commission d'Etablissement des Methodes d'Analyse Commissariat a l'Energie Atomique BP 6, 92265, Fontenay aux Roses (France)
- (4) AEA Fuel Services, Chemistry Division, Harwell Laboratory, Oxfordshire OX11 0EA (UK)
- (5) New Brunswick Laboratory US Department of Energy 9800 South Cass Avenue Argonne, IL 60439-4899 (USA)
- (6) All Union Foreign Economic Association "Techsnabexport", Staromonetnyi Per. 26, 109180, Moscow (USSR).

The IAEA AQCS Programme provides three main types of material.

- Materials that can be used in analytical laboratories working in the fields of nuclear technology and isotope hydrology. These include uranium ore reference materials and other substances relevant to nuclear fuel technology as well as stable isotope reference materials for mass spectrometric determination of isotope ratios in natural waters.
- Materials with known contents of uranium, thorium and/or transuranic elements or fission products for the determination of environmental radioactivity or control of the control nuclear safety.
- Materials for use in the determination of stable trace elements in environmental or biomedical research. Radiochemical methods such as neutron activation or isotope dilution analysis, are often used in the determination of such trace elements and constitute an important contribution of nuclear techniques to applied science.

Table 9.10 lists the radionuclides referenced by IAEA, their activity, matrix, and sample code. Table 9.10 includes also materials of marine origin [62]. The intercomparison samples cover a range of materials and contain radionuclides with very different levels.

TABLE 9.10

RADIONUCLIDES REFERENCED BY IAEA

Referenced analyte	Activity or concentration (Bq/kg)	Confidence	Matrix interval	Reference date	Sample code
^{40}K	391	379–405	Tuna homogenate, Mediterranean	1 January 1989	Iaea-352
	527	510–543	Milk powder	31 August 1987	A-14
	150	141–161	Sea-plant, <i>Posidonia oceanica</i>	1 January 1988	IAEA-307
	220	189–226	Sediment, marine	1 January 1985	SD-N-2
	240	211–269	Sediment, lake	31 January 1986	SL-2
	272	252–299	Fish flesh	1 January 1986	MA-B-3/RN
	539	510–574	Milk powder	31 August 1987	IAEA-152
	552	563–569	Milk Powder	1 January 1990	IAEA-321
	657	637–676	Clover	1 August 1986	IAEA-156
	1381	1320–1456	Seaweeds, Mediterranean	1. January 1988	IAEA-308
	1575×10^3	1511–1644	Hay powder	31 August 1987	IAEA-154
^{90}Sr	1.5	1.33–1.57	Milk powder	31 August 1987	A-14
	3.3	3.16–3.44	Milk powder	1 January 1990	IAEA-321
	6.9	6.0–8.0	Hay powder	31 August 1987	IAEA-154
	7.7	7.0–8.3	Milk powder	31 August 1987	IAEA-152
	14.8	13.4–16.3	Clover	1 August 1986	IAEA-156
	30.34	24.2–31.67	Soil	30 January 1983	SOIL-6
	54.8	46.3–59.2	Bone, animal	15 December 1981	A-12
^{106}Ru	23	22–25	Seaweeds, Mediterranean	1 January 1988	IAEA-308
	33.5	30.0–36.5	Sea-plant, <i>Posidonia oceanica</i> 40	1 January 1988	IAEA-307
$^{110\text{m}}\text{Ag}$	20	1.2–27	Seaweeds, Mediterranean	1 January 1988	IAEA-308
	5.1	4.8–5.5	Sea-plant, <i>Posidonia oceanica</i>	1 January 1988	IAEA-307

TABLE 9.10 (continued)

Referenced analyte	Activity or concentration (Bq/kg)	Confidence interval	Matrix interval	Reference date	Sample code
^{134}Cs	1.6	1.5–1.8	Seaweeds, Mediterranean	1 January 1988	IAEA-308
	1.6	1.5–1.9	Sea-plant, <i>Posidonia oceanica</i>	1 January 1988	IAEA-307
	15.5	14.8–16.2	Milk powder	1 January 1990	IAEA-321
	132	126–138	Clover	1 August 1986	IAEA-156
	764	722–802	Milk powder	31 August 1987	IAEA-152
	1355	1295–1417	Whey powder	31 August 1987	IAEA-154
^{137}Cs	2.7	2.5–2.8	Tuna homogenate, Mediterranean	1 January 1989	IAEA-352
	0.8	0.5–1.0	Sediment, marine	1 January 1985	SD-N-2
	1.79	1.62–1.97	Milk powder	31 August 1987	A-14
	2.4	2.2–2.6	Sediment lake	31 January 1986	SL-2
	4.9	4.5–5.2	Sea-plant, <i>Posidonia oceanica</i>	1 January 1988	IAEA-307
	5.6	5.3–6.0	Seaweeds, Mediterranean	1 January 1988	IAEA-308
	14.2	13.7–15.3	Fish flesh	1 January 1986	MA-B-3/RN
	53.65	51.43–57.91	Soil	30 January 1983	SOIL-6
	72.6	71.1–74.2	Milk powder	1 January 1990	IAEA-321
	264	254–274	Clover	1 August 1986	IAEA-156
	2159	2503–2209	Milk powder	31 August 1987	IAEA-152
	3749	3613–3887	Hay powder	31 August 1987	IAEA-154
^{210}Pb	0.6	0.36–1.0	Tuna homogenate, Mediterranean	1 January 1989	IAEA-352
	73	66–75	Seaweeds, Mediterranean	1 January 1988	IAEA-308
^{210}Po	2.2	1.7–2.7	tuna homogenate, Mediterranean	1 January 1989	IAEA-352
^{226}Ra	3.1	21–4.4	Sea-plant, <i>Posidonia oceanica</i>	1 January 1988	IAEA-307

TABLE 9.10 (continued)

Referenced analyte	Activity or concentration (Bq/kg)	Confidence	Matrix interval	Reference date	Sample code
	5.2	4.4–6.7	Bone, animal	15 December 1981	A-12
	79.92	69.56–93.43	soil	30 January 1983	SOIL-6
	269	250–287	soil	30 January 1988	IAEA-312
	342	307–379	sediment, stream	30 January 1988	IAEA-313
	732	678–787	Sediment, stream	30 January 1988	IAEA-314
^{228}Th	25	2.2–3.6	Seaweeds, Mediterranean	1 January 1988	IAEA-308
$^{232}\text{Th}^a$	4.9	4.5–5.4	Sediment, marine	1 January 1985	SD-N-2
^{238}Pu	0.017	0.016–0.023	Seaweeds, Mediterranean	1 January 1988	IAEA-308
	0.025	0.022–0.028	Sea-plant, <i>Posidonia oceanica</i>	1 January 1988	iaea-307
^{239}Pu	1.04	0.962–1.11	Soil	30 January 1983	SOIL-6
^{239}Pu ^{240}Pu	8.8	6.51–4.0	Sediment, marine	1 January 1985	SD-N-2
	0.50	0.46–0.52	Seaweeds, Mediterranean	1 January 1988	IAEA-308
	0.72	0.66–0.79	Sea-plant, <i>Posidonia oceanica</i>	1 January 1988	IAEA-307
^{241}Am	0.036	0.030–0.050	Sea-plant, <i>Posidonia oceanica</i>	1 January 1988	IAEA-307
	0.17	0.16–0.25	Seaweeds, Mediterranean	1 January 1988	IAEA-308

^aNote: the ^{232}Th is in equilibrium with ^{228}Ra and ^{228}Th .

IAEA intercalibration exercises are conducted with the involvement of many laboratories. As an example, Fig. 9.4 shows the results of an intercomparison run for ^{137}Cs determination in milk powder. Some laboratories had difficulties in determining the activity level. This situation is rapidly improving with time: Table 9.11 shows the improvements in the quality of the work at the participating laboratories. This is also seen in Table 9.12 where the mean values and relative standard deviations of three intercomparison runs for the ^{90}Sr determination in simulated air filters are presented.

The intercomparison exercises show a need for greater standardization of the analytical techniques used for radionuclide determination. This is indicated in ref. [63], where the

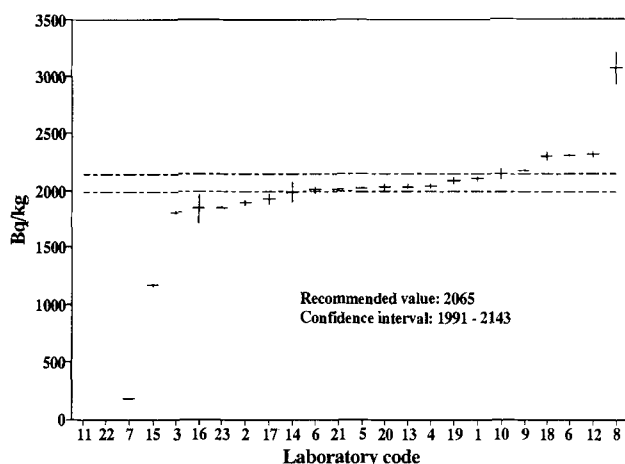


Fig. 9.4.

bias and measurement errors in radioactivity data from four European radiation research laboratories were reported.

Within the framework of the International Chernobyl Project, the IAEA's Seibersdorf Laboratories organized an intercalibration exercise [64] among some of the laboratories which were involved in assessing the environmental contamination in the former USSR by the accident. The objective was to assess the reliability of the radioanalytical data for food and environmental samples, which were used to assess the doses. The initial study reference materials from the stocks of the IAEA's Analytical Quality Control Service (AQCS) were re-labelled and submitted to 71 laboratories as blind samples. These natural matrix materials included samples of milk (containing two different levels of radioactivity), soil, air filters and clover. The concentrations of radionuclides in these samples were known from previous intercalibration exercises.

The overall range in performance was broad, which is as observed in previous international intercomparisons. This is illustrated in Table 9.13 where the results of original IAEA intercomparison run (worldwide) and former Soviet Union laboratories, for high level (H) milk are presented.

TABLE 9.11

DETERMINATION OF ^{137}Cs IN THE SAME MILK POWDER DURING INTERCOMPARISONS IN 1983 AND 1989

	Mean value (Bq/kg)	Rel. SD (%)	% outliers/lab.
1983	2.08	45	10
1989	1.70	19	0

TABLE 9.12

DETERMINATION OF ^{90}Sr IN SIMULATED AIR FILTERS DURING INTERCOMPARISON RUNS IN 1973, 1976, 1988

	Mean value (Bq/filter)	Rel. SD (%)
1973	178	27
1976	179.5	17
1988	231	3

9.13. NON-RADIOMETRIC METHODS

There is a variety of situations in which it is better to determine the concentration of a radionuclide by a mass measurement rather than by measuring the activity present. This approach is possible using a wide range of instrumental methods of non-radiometric elemental analysis; analytical measurements can be performed also by element-specific chemical methods, some of which are extremely sensitive.

The most important criterion for selecting an analytical method is whether the technique is sufficiently sensitive to measure the amount of radionuclide present in the sample. This is a very different problem when considered from the viewpoint of analytical chemists who use radiometric methods and those who use non-radiometric methods. Limits of detection in radiometric methods can be as low as 10^{-4} Bq, although, ~ 1 mBq is a more generally attainable detection limit. For non-radiometric methods, the detection limit is expressed in terms of mass and the relationship between radiometric and non-radiometric limits of detection will depend upon the half-life of the radionuclide of interest.

Table 9.14 (from ref. 65) shows the mass corresponding to 1 mBq for a number of important radionuclides. Although 1 mBq of ^{232}Th is readily measured by a number of non-radiometric methods, 1 mBq of ^{137}Cs could only be detected by the most sensitive of methods and is probably best determined radiometrically.

TABLE 9.13

COMPARISON OF PERFORMANCE OF THE TWO GROUPS OF LABORATORIES:
WORLDWIDE VERSUS FORMER SOVIET UNION

Radionuclide	Range of reported values for milk(H) (Bq/kg)	
	Worldwide	USSR
^{37}Cs	469.3 – 2491.3	175 – 3070
^{34}Cs	58.0 – 652.5	184.7 – 542.5
^{40}K	103.6 – 3650.0	429 – 4959
^{90}Sr	5.53– 8.54	1.43– 68.8

TABLE 9.14

THE MASS OF 1 mBq FOR A SELECTION OF RADIONUCLIDES WITH A VARIETY OF HALF-LIVES (after ref. 64)

Radionuclide	Half-life (years)	Mass of 1 mBq (g)
^{232}Th	1.4×10^{10}	2.5×10^{-7}
^{238}U	4.5×10^9	8.1×10^{-8}
^{129}I	1.7×10^7	1.6×10^{-10}
^{99}Tc	2.1×10^5	1.6×10^{-12}
^{239}Pu	2.4×10^4	4.3×10^{-13}
^{14}C	5.8×10^3	6.1×10^{-15}
^{137}Cs	30	3.1×10^{-16}

Non-radiometric methods offer a variety of features and their use may be favoured for reasons other than improved sensitivity or isotopic selectivity. They can, in some instances, be used to perform analyses with less sample preparation and greater speed or sample throughput, and allow remote analysis or provide elemental or isotopic maps or depth profiles [65].

The instrumental methods of elemental analysis can be conveniently grouped as below.

- (i) Methods based on X-ray spectrometry
 - X-ray fluorescence analysis (XRF)
 - Total reflectance X-ray fluorescence analysis (TXRF)
 - Electron microprobe analysis (EMA)
 - Particle induced X-ray emission (PIXE)
 - Synchrotron radiation induced X-ray emission (SRIXE)
- (ii) Methods based on ultraviolet or visible spectroscopy
 - Atomic absorption spectroscopy (AAS)
 - Graphite furnace AAS (GFAAS)
 - Atomic fluorescence spectroscopy (AFS)
 - Inductively-coupled-plasma optical-emission spectroscopy (ICPO-ES)
 - Glow-discharge optical-emission spectroscopy (GD-OES)
 - Laser-excited atomic-fluorescence spectroscopy (LEAFS)
 - Laser-induced-breakdown spectroscopy (LIBS)
 - Resonance-ionization spectroscopy (RIS)
- (iii) Methods based on mass spectrometry
 - Spark-source mass spectrometry (SSMS)
 - Glow-discharge mass spectrometry (GDMS)
 - Inductively coupled-plasma mass spectrometry (ICP-MS)
 - Electro-thermal vaporization-ICPMS (ETV-ICP-MS)
 - Thermal-ionization mass spectrometry (TIMS)
 - Accelerator mass spectrometry (AMS)

Secondary-ion mass spectrometry	(SIMS)
Secondary neutral mass spectrometry	(SNMS)
Laser mass spectrometry	(LMS)
Resonance-ionization mass spectrometry	(RIMS)
Sputter-initiated resonance-ionization spectroscopy	(SIRIS)
Laser-ablation resonance-ionization spectroscopy	(LARIS)

McMahon [65] has reported on intercomparison of non-radiometric methods for the measurement of low levels of radionuclides. He has classified the above analytical techniques according to the amount of isotopic information and the amount of sample required. The conclusions are presented in Table 9.15.

Two techniques are discussed in some detail: ICP-MS and AMS.

9.13.1. ICP-MS

As mass spectrometry has continued to gain sensitivity and reliability, inductively coupled plasma/mass spectrometry (ICP-MS) has become increasingly useful in the measurement of radionuclides. The optimization of ICP-MS is improving our ability to use the atomic detection of radionuclides in that it allows the near-complete isotopic analysis of any form of sample. Aqueous samples are generally introduced into the plasma source, and solids or individual particles, and organic solutions, may be atomized and continuously introduced into the plasma source.

ICP-MS sensitivity, which is currently $\sim 8 \times 10^9$ atoms, can be improved by:

- the use of more efficient sample introduction techniques,
- understanding of the basic principles of ion and gas dynamics in the ICP-MS interface,
- and the use of high-resolution mass spectrometers with high ion transmission.

The ultimate sensitivity could approach $\sim 10^7$ atoms, which would result in a superior detection capability for all radionuclides with half lives greater than 1 year.

TABLE 9.15

ANALYTICAL TECHNIQUES CLASSIFIED BY AMOUNT OF ISOTOPIC INFORMATION AND AMOUNT OF SAMPLE REQUIRED (after ref. 64)

	Bulk samples	Small samples profiling	Imaging and depth
No isotopic information	ICP-OES	XRF, GFAAS, LEAFS, TXRF	PIXE, SRIXE
Minor isotope determination	ICP-MS, GDMS, SSMS	ETV-ICP-MS	SIMS, SNMS, LMS, SIRIS
Trace isotope determination		TIMS, RIMS, AMS	

9.13.2. AMS

For isotopes with long life times, >1 year, it may often be more advantageous to use atom-counting techniques rather than traditional decay-counting methods. This is especially true for measurements where efficiency is a criterion, as for small samples, or if high precision is required. While atom counting has a counting rate that is essentially independent of decay lifetime and sample size, the decay-counting rates are comparable only if the isotopic half-life is less than one year for a sample size of the order of 1 mg. Of course, if sufficient material is available, the decay counting rate can always be improved by using more material [66].

Accelerator mass spectrometry (AMS) extends the capabilities of atom-counting using conventional mass spectrometry, by removing whole-mass molecular interferences without the need for a mass resolution very much better than the mass difference between the atom and its molecular isobar. This technique has been used with great success for the routine measurement of ^{14}C , ^{10}Be , ^{26}Al , ^{36}Cl and, recently, ^{129}I . Analysis of ^{14}C by AMS can, for example, generate dates with a precision that is at least equal to the best conventional beta particle counting facility. In many cases, where small sample analysis is required, the AMS method has proved superior [67]. A complete description of AMS can be found in review articles [68,69] or recent conference publications. The application of AMS to ^{129}I measurement has been discussed in detail in reference [66].

Six long-lived radionuclides beyond uranium exist which have half-lives greater than 100 ka (^{236}Np , ^{237}Np , ^{242}Pu , ^{244}Pu and ^{248}Cm). The first two are natural by-products of the nuclear industry. Nuclear-weapons tests will generate the plutonium and curium isotopes although attempts have been to detect presolar system ^{244}Pu in ores [70] or ^{244}Pu from more recent supernova debris. The detection of these isotopes is still in the development stage. Unlike the natural elements, isobaric interferences are not a major problem as all isotopes will be equally rare or non-existent because of their very short decay half-lives compared to the lifetime of the solar system.

9.14. CONCLUSIONS

Many radionuclides, both natural and man made, are distributed through all the compartments of the environment. They contribute to the dose received by man either by inhalation, digestion or direct exposure. Therefore, it is of the utmost importance to develop experimental procedures capable of measuring even minute activities of all radioisotopes.

In this chapter, we have shown that such capabilities exist and that the progress in this field will result in reduced minimum detection limits. An important aspect of this type of measurement is quality control, which has been receiving increased attention.

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Chapter 10

Quality assurance in environmental analysis

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10.1. INTRODUCTION

Human activities have a considerable bearing on the biosphere. The impacts are often brought about by discharges of chemicals whose nature and effects vary considerably. Some compounds are emitted in sufficient quantities to perturb global chemical equilibria. Prime examples are provided in the field of atmospheric pollution. The concentrations of carbon dioxide, methane and chlorofluorocarbons in the stratosphere are enhanced and give rise to the greenhouse effect. The present levels of emissions could lead to a significant rise in the mean global temperature, thereby inducing changes in the global climate and its distribution, with large socio-economic and ecological implications [1]. Compounds such as chlorofluorocarbons also contribute to the degradation of the ozone layer, giving rise to an increase in the intensity of UV radiation on the surface of the earth. Chemicals may also primarily affect biotic equilibria, with the eutrophication of fresh and marine waters by, for example, nitrates and phosphates serving as an illustration. The inputs of large quantities of nutrients into the aquatic environment result in an increased growth of algae which, in turn, modifies the population structure of the ecosystems. Oxygen deficiencies may occur in water systems because of the mineralization of algae. Substances that are toxic for biota and for human beings constitute a third class. Lists of priority pollutants have been compiled by individual countries and, for example, by the European Community. Compounds on these lists have a high priority in environmental

management and legislation. Examples of such chemicals are heavy metals such as cadmium and mercury, lipophilic organic compounds such as polychlorobiphenyls, polycyclic aromatic hydrocarbons, dioxins, and several agrochemicals.

Environmental management depends on information on a wide range of compounds. Unreliable or inaccurate information may lead to large economic losses, such as sanitation measures which may be taken needlessly, or to unacceptable risks for the environment or human health if necessary measures are not taken. It is essential that the quality of information on the environment is adequate.

Quality assurance of environmental analyses represents an aspect in the quality management of the overall process that provides information. The discussion of quality assurance of environmental analysis is therefore put into a broader context. In Section 2, a brief analysis of the information requirement is presented. The assessment of this information requirement is the start of a chain of activities that generates the information pursued. This process is discussed in Section 3. Sections 4 and 5 deal with quality management in the laboratory. Section 4 treats the main features of the GLP principles, ISO Guide 25 and the standard EN 45001. Section 5 focuses on technical aspects. Section 6 deals with interlaboratory studies.

10.2. ENVIRONMENTAL MANAGEMENT AND THE NEED FOR INFORMATION

The need for information on chemical substances in the environment has a dynamic nature. New types of compounds are continually being developed, produced and used for a variety of applications. Some of these substances will require profound study, possibly leading to measures to control or limit their use. The use of certain chemicals may diminish, for example owing to the introduction of new technologies, a reduction in demand for the product concerned, or as result of actions taken in the context of environmental legislation. The need for information about the presence of these chemicals in the environment may decrease concurrently.

A discussion on the need for information about chemical substances can be approached in a systematic manner, employing the concept of the "policy life cycle" [2] (see Fig. 10.1).

Four phases are distinguished. In the first phase, it is recognized that a particular situation constitutes an environmental problem. Signals from research institutes, universities and so on may point to a certain problem. These signals can trigger more systematic investigations. Laboratory studies may be carried out to establish the ecotoxicological and physical-chemical properties of the compounds, with surveys to examine the sources, spatial distribution and pathways through the environment. This information is used to assess the necessity of taking measures.

In the second phase, a policy is developed to solve and control the problem. Measures will be taken to reduce the inputs into the environment. Often, standards will be set for the concentrations of the chemicals in products, environmental compartments (air, water, sediment, soil), waste waters and waste materials.

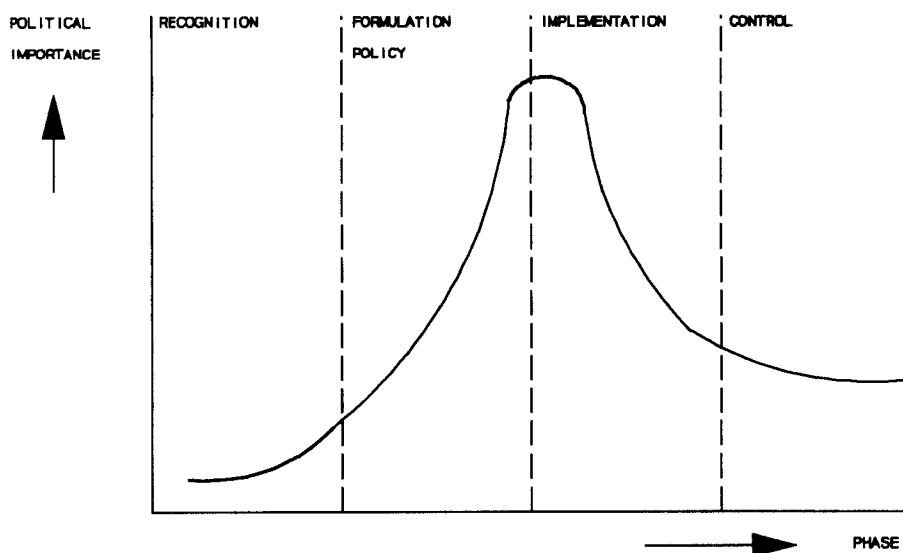


Fig. 10.1. The policy life cycle.

The third phase entails the implementation of the policy. Chemical measurements are necessary for several purposes:

- Legislation may require the checking of the concentrations with respect to the standards in products, environmental compartments and/or in waste materials. Corrective actions are necessary if the standards are exceeded.
- Legislation may entail that a levy is imposed, on the basis of the concentrations of a chemical parameter (e.g., the chemical oxygen demand in waste waters).
- The method of disposal for a certain material (e.g. dredged material) may have to be established on the basis of its degree of contamination. In the Netherlands, for example, the waterways have to be dredged regularly to maintain the depth required for shipping. The sediments are quite contaminated. The method of disposal of the material is assessed on the basis of the concentrations of a number of priority pollutants.
- Environmental monitoring programmes may be started to assess temporal trends and spatial distributions. These programmes provide feedback with respect to the effectiveness of the measures taken.

In the fourth and last phase of the policy life cycle the policy objectives will have been reached. The measurements have acquired a routine nature. Temporal trend monitoring can be terminated or replaced by programmes which check whether the situation remains under control.

The information-need changes through the respective policy phases. Each information-need poses specific requirements for the design of the programme of measurements, the performance characteristics of the methodology, and the evaluation and presentation of data. In principle, the concept of quality should be considered in this context. The quality of the information is high if it meets the needs and is acquired in a cost-effective manner.

Particularly from the second stage onwards, the number of laboratories performing the measurements will increase. Often, a development arises for which a few "expert" laboratories develop methodology and carry out inventories. The number of laboratories involved expands as the importance of the subject grows. It can be expected that the increase in active laboratories will be accompanied by a reduction in overall performance. When analytical methodology is fully in place, i.e. when well-validated, harmonized methodologies, reference materials and interlaboratory studies are available, the overall performance will improve.

Environmental management increasingly takes measures to prevent problems and laboratory tests are necessary in this context. For example, most industrialized nations require information on a range of environmental properties (e.g. physico-chemical properties, and toxicity, biodegradability, carcinogenic and mutagenic properties) for new chemicals which are brought onto the market. The tests have to be conducted in compliance with GLP guidelines.

Environmental issues have to be seen more and more in an international context. Standards for the concentrations of contaminants in products or in environmental compartments are harmonized internationally. Monitoring programmes increasingly involve international cooperation. This development implies that laboratory data have to be internationally comparable. To this end, refinement of methodology has to be achieved, with proper arrangements for traceability on an international level. Comparability of data does not, however, automatically imply that the desired information is actually acquired. For example, internationally coordinated monitoring programmes also require fine tuning of the design of the programmes, including agreement on such basic elements as the parameters, environmental compartments of measurement, definitions, data formats for reporting and the performance characteristics that the methodology has to meet. A holistic approach to quality management is necessary [3,4].

10.3. TOTAL QUALITY MANAGEMENT OF ENVIRONMENTAL INFORMATION

The process for the acquisition of information is schematically outlined in the quality circle depicted in Fig. 10.2 [5]. The figure and the following discussion are based on the standard ISO 9004 [6].

The first step is to analyze critically the information needs and expectations [7]. It is not sufficient to specify which parameters have to be measured in which matrices. Where possible, quantitative criteria should be established: for example, the minimum temporal trend which should be detected ($x\%$ difference in y years with a probability of $z\%$) or limits for the type I and type II errors when compliance with standards has to be checked. The assessment should include such aspects as the time schedule and data management, and the way in which the information must be presented. This step should result in qualitative and quantitative specifications that the information has to meet.

These specifications are put into the design phase. The physico-chemical properties of the chemicals have to be considered for selection of the most appropriate compartment for measurement [8] and to establish proper procedures for sampling, preservation and

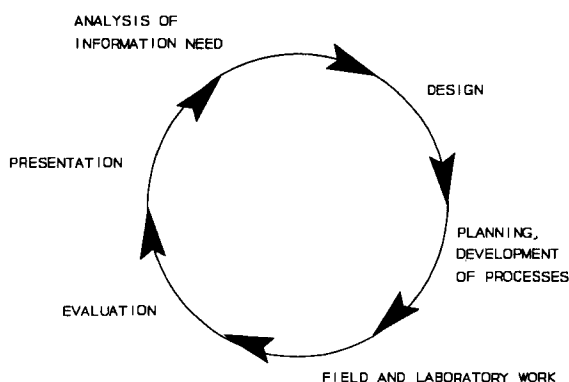


Fig. 10.2. A quality circle for the process of generating environmental information.

laboratory analysis. Knowledge of chemical, biological and physical processes, of specific local characteristics or properties of the object of investigation, and of the analytical methodology and statistics should be used in devising a programme of measurements. Environmental modelling can provide an important contribution in this respect. Data management has to be accounted for. Quality assurance for field and laboratory work should be considered, including the assessment of the quality criteria the activities must meet. Acceptance criteria for the results have to be established.

The planning phase includes the drafting of protocols for sampling and analysis, and the provision of instructions and requisites to the personnel and laboratories involved. Important factors pertinent to sampling are the presence of a fully documented sampling plan, a system for unambiguous sample identification and arrangements for quality assurance of the sampling [9,10]. The sample plan should include the objectives, determinands, sampling locations, number and types of samples, identification of samples, reference to standard operating procedures for sampling and preservation, and required field information (time, weather conditions, depth and so on). Often, a scheme of inspections can be devised for quality assessment of the sampling.

All field measurements and "administrative data" concerning the samples should be noted in logbooks. The sample identification should be such that a proper traceability is assured from sampling to reporting of the data.

When work is contracted out, the requirements regarding methodology and quality assurance should be specified.

The field and laboratory work should be carried out within a quality system (e.g. EN 45001 [11], ISO 25 [12]). A more detailed discussion of such systems is given in the next section.

Evaluation of the laboratory data constitutes the next phase. The data should be assessed critically and their plausibility checked. The evaluation should invoke the expertise that was used to design the programme of measurements.

Presentation of the results ends a cycle. The presentation should focus on the information need, and be clear and timely.

Every stage should include procedures to verify that the activities have been carried out in conformance with previously defined quality criteria. Evaluation of the whole cycle, and of the experiences of the users of the information, provides feedback which can be used to improve the process. In this manner, the quality of the information generating process is continually maintained and systematically improved.

In the approach described in this section, the whole process from the analysis of the need for information to the presentation of results is subject to quality management. The information is the result of a chain of activities, and its quality will depend on the strength of the weakest link. Quality control and assurance in the analytical laboratory form a subset of a larger scheme. Quite often, however, a laboratory is commissioned to carry out only a part of the process depicted in Fig. 10.2. In this case, good communication between the (analytical) laboratory and the customers is essential. Laboratories have to ensure that their methodology meets the requirements put forward in the objectives of the measurement. The expertise of laboratories can be conferred as a service, for example in the selection of the sampling methodology, sample preservation and analysis, and design of the sampling programme.

10.4. QUALITY SYSTEMS FOR CHEMICAL ANALYSIS

At present, quality assurance is an important issue in all sectors of industry. Literature on quality management in general is widely available. For laboratories working in the environmental field, the principles of Good Laboratory Practice and the quality systems delineated in the ISO Guide 25 and the European standard EN 45001 are of particular importance. Some papers on quality assurance are given in references [9,13–20].

The concept of Good Laboratory Practice (GLP) was introduced by the US Food and Drug Administration (FDA) in the mid-1970s. This organization observed serious shortcomings during the examination of toxicological tests conducted by animal testing laboratories. These shortcomings comprised [21]:

- poorly conceived, carelessly executed, inaccurately analyzed or reported experiments;
- lack of awareness on the part of technical personnel of the importance of protocol adherence;
- inaccurate observations, record keeping and record transcriptions;
- failure of management to assure critical review of data or proper supervision of personnel;
- use of poorly qualified or poorly trained personnel;
- disregard for proper laboratory animal care and data management procedures;
- failure to monitor studies performed, in whole or in part, by contract laboratories;
- lack of verification of the accuracy and completeness of scientific data;
- deliberate falsification of data by management and/or laboratory personnel.

In response to these observations, the FDA established the principles of GLP which were accepted in the United States in 1976. The importance of the GLP guidelines was acknowledged by the Organization for Economic Cooperation and Development (OECD). The OECD published similar guidelines in 1982, which are applicable for all toxicologi-

cal research carried out for the risk assessment of chemicals for man and environment [22,23].

GLP has the objective of assuring the quality of laboratory data and achieving transparency of the design, conduct and evaluation of the study. GLP provides criteria for the organization of a laboratory and for the way it should work in order to obtain valid data. It does not prescribe specific methodology. The criteria enable third parties to reconstruct the approaches and methods used and the results obtained. Most industrialized nations have incorporated the use of GLP guidelines in their legislation [23].

GLP requires that the organization is mapped and that the responsibilities of staff members, analysts and technicians are documented. The laboratory management has to ensure that the qualifications of the personnel are appropriate to the complexity of the work. Important items are the appointment of a study director for each project and the presence of a quality assurance unit (QAU). The study director has to make up a study plan for the project and supervises its execution. On completion of the study, the study director has to prepare a final report.

The QAU implements and maintains the quality assurance programme. The QAU staff check whether the tests are carried out according to GLP guidelines. The final report is thoroughly assessed by the QAU and has to be signed by a member of the QAU staff to confirm that the study has been conducted in compliance with GLP guidelines. Members of the QAU should have no involvement in the studies that they assess.

GLP requires a careful documentation of the activities. Standard operating procedures (SOPs) are to be used for procedures that are used frequently (analytical methodology, reporting of data, sample handling, etc.). Activities of a non-repetitive nature are to be documented in the study plan. The raw data of tests must be recorded. When changes are made, the original data have to be saved. Likewise, changes in the study plan must be recorded. GLP requires that all documentation, reports etc. are signed by those responsible.

The location, construction and spatial planning of the laboratories should meet the needs for performing the investigations correctly, without contamination and without danger to the health of the personnel. Instruments must be checked and maintained at regular intervals. A record has to be kept of these activities.

Appropriate measures have to be taken for the storage of chemicals. Chemicals have to be adequately labelled. Important items include the supplier, the purity, special storage instructions, stability, concentration and toxicity. GLP also imposes requirements on the storage of records and specimens.

ISO Guide 25 and the European Standard EN 45001 provide a basis for the establishment of quality systems in calibration and testing laboratories. The criteria delineated in GLP and ISO 25/EN 45001 resemble each other for several topics, e.g. personnel, facilities, the use of SOPs and the management of equipment and chemicals. In ISO 25 and EN 45001 no reference is made to the study director, as this task is not relevant for a routine testing laboratory. However, ISO 25 and EN 45001 elaborate considerably upon the technical contents. Methods have to be properly validated. Their performance has to be monitored with an ongoing quality control scheme. Specific attention is paid to calibration and measurement traceability. Certified reference materials are to be used wherever possible. Participation in laboratory performance studies is strongly encouraged as a means for the assessment of accuracy and for quality control/assessment purposes. ISO 25

and EN 45001 also contain criteria for computers and computer controlled systems in laboratories. A joint WELAC/EURACHEM Working Group has prepared a very useful paper with guidance for the interpretation of EN 45001 and ISO 25 [20].

GLP and ISO 25/EN 45001 differ in approach. The purpose of the OECD principles of Good Laboratory Practice is described as promoting the development of quality test data. GLP does not make reference to a quality system as delineated in ISO 8402 [24]. Instead, a quality assurance programme is introduced which is defined as an internal control system designed to ascertain that the study is in compliance with the GLP principles. ISO 25 and EN 45001 provide criteria for the establishment of a quality system. The top management has to set out a quality policy in which it expresses the objectives, expectations and commitments. Resources have to be made available to develop and implement a quality system. The latter task is allocated to a quality manager. The system is to be documented in a quality manual. The contents of this manual are explicitly stated in ISO 25.

In summary, GLP focuses on quality control of the study, whereas ISO 25 and EN 45001 entail active quality management in laboratories.

Laboratories which have to conform to the GLP principles are inspected biannually. Usually, a general inspection of the laboratory is conducted in combination with audits of a number of randomly selected studies. GLP inspections do not consider the design of the study, the methodology employed or the interpretation of the results, but assess whether the studies have been carried out in compliance with the GLP criteria (e.g. the qualifications of personnel and documentation of the study).

Laboratories that have implemented a quality system according to ISO Guide 25 or EN 45001 can apply for accreditation. In Western Europe, accreditation bodies cooperate in the Western European Laboratory Accreditation Cooperation (WELAC). WELAC has the objective of harmonizing Western European accreditation practices and establishing a scheme of mutual agreements, so that an accreditation granted by an affiliated body is recognized by all other bodies that have entered the agreement scheme. Worldwide harmonization of accreditation practices is pursued by the International Laboratory Accreditation Conference (ILAC).

Laboratories can have several reasons for acquiring an accreditation. Accreditation strongly enhances their competitiveness as it provides confidence in their data. Increasingly, customers require laboratories to be accredited. Also in the context of environmental legislation, accreditation may become mandatory.

Laboratories seeking accreditation can obtain information about the procedures and guidelines for the interpretation of the criteria from the accreditation body. Application is achieved by providing this body with an application form, a copy of the quality manual and a list of the determinands along with basic information about the methodology. The accreditation body forms an assessment team for inspection if the information provided by the laboratory is adequate. The team is composed of experts on quality systems and on methodology. The design and operation of the quality system is investigated. The methodology is thoroughly assessed, paying attention to validation, quality assessment measures, etc. It is checked that the stated performance characteristics are met. After accreditation has been granted, surveillance visits are conducted yearly. A full reassessment takes place after every fourth year [13].

10.5. A CLOSER LOOK INTO QUALITY ASSURANCE IN THE LABORATORY

10.5.1. Organization and management

The senior management of a laboratory fulfils an important role in quality management. The quality policy should provide clear guidance with respect to the quality objectives and thus head the personnel to the proper direction. W.E. Deming, one of the founders of quality management, stressed the importance of a stimulating and motivating management. He pointed out the need for a good education programme, warned against parochialism, and made a plea for an atmosphere in which personnel could express themselves. These points have also been stressed by Griepink [17].

10.5.2. Development of methodology

ISO Guide 25 and EN 45001 do not consider any methodological details. A high level of professional expertise embedded in a quality system conforming to these standards forms the basis for a good output. Quality has to be built into methodology during the development stage. The entire analytical process, including sampling, preservation, sample pretreatment and work-up, instrumental analysis and data handling has to be scrutinized.

Contamination, or loss of analyte may occur at any step. Four principal sources of problems with the analytical blank can be distinguished: contamination may arise from the environment in which the work is carried out, from the reagents, from the instruments, and from the performance of the technician [25]. Lack of contamination control is often identified as a principal source of error in environmental analysis [37,38]. Several causes may be identified for losses of analyte during the analytical process: adsorption of the analyte to, e.g. storage vessels; degradation of the analyte owing to, e.g. biodegradation; photophysical or photochemical reactions; thermal lability or chemical reactivity (e.g. [26]); volatilization of the analyte; and the performance of the technician. The extent of contamination and of losses during the analytical process has to be acceptable in relation to the analyte concentration in the sample and has to be controlled.

Possible sources of bias should be critically assessed. Bias may arise from instability of samples between sampling and analysis, improper calibration, improper blank corrections and the inability to determine all forms of the determinand [27].

Calibration has been identified as a major source of systematic error in environmental analysis. An inter-laboratory study, in which a standard solution of chlorobiphenyls was analyzed, revealed an inter-laboratory variance of 24% [28]. An inter-laboratory study of nutrients in sea-water showed that the analytical sensitivity and precision were adequate, but that calibration problems occurred [29]. Poor quality of standards has been encountered in inter-laboratory studies of trace metals in biological tissues [20] and in estuarine water [30], of chlorobiphenyls in marine media [31], and of polycyclic aromatic hydrocarbons [32]. Errors related to calibration include improper purity, stoichiometry or identity of compounds, dilution errors, the neglect of the density of solvents and of temperatures when preparing standard solutions on a volume basis, inadequate internal

standards or errors in their application and bad storage and handling of stock solutions [33,34].

The quality of commercially available standard solutions can be questionable [35]. It is good practice to check the contents of such solutions by using an independent standard prepared from pure or stoichiometric compounds [13,34]. The exchange of calibration solutions with other laboratories can prove to be highly instructive.

Frequently, dilution, weighing or calculation errors are made in the preparation of standard solutions. Each fresh bottle of standard solution should be checked by comparison with the old bottle. It has been recommended that one should not prepare a calibration curve by serial dilution from a single solution. The chance of unnoticed mistakes can be reduced by using two or more starting solutions [13].

A thorough assessment of the response curve of the detector in the working range should be carried out. Bracketing calibration is preferred. The concentrations of the calibrants should be optimized so that the calibration curve is well established. The working range established in this manner should not be exceeded [13].

The analytical blank should be under control. Blank determinations should be carried through the entire analytical procedure. Insight into the actual sources contributing to the blank provides guidance for reducing (if necessary) and controlling it. The blank signal should be low in comparison with the signal of the analyte. Control of the blank is a prerequisite in procedures for blank correction. Statistical considerations in blank corrections have been presented by Taylor [36].

Difficulties connected with the inability to determine all forms of the determinand are illustrated with examples from inorganic and organic trace analysis. Trace metal analysis often invokes techniques such as AAS, ICP or voltammetry. In these cases, samples often have to be digested to isolate the metals from the matrix. The procedure for digestion has to take into account the nature of the matrix, the properties of the element and the technique used for quantification. This can be illustrated for biological materials.

The digestion of biological materials can be accomplished at normal pressure or at elevated temperatures and pressures with, for example, nitric acid. This procedure does not decompose all organic material, but the degree of digestion is sufficient for the analysis of a range of metals using AAS or ICP. Voltammetric techniques, however, are sensitive to the form in which the metal is present. Complexing agents in the digest may result in metal complexes that are not electrolytically active. The complete digestion of organic material is required, for example by high pressure ashing or by an additional treatment of the sample solution with perchloric acid [37].

More vigorous digestion procedures may also be required in order to decompose stable metal compounds. An example is provided by the determination of arsenic in biological tissues. In an inter-laboratory study of trace metals in biological tissues, it was observed that arsenic exhibited the highest lack of comparability. Wet-ashing techniques employing strong oxidizing agents (e.g. HNO_3 with H_2O_2) appeared to give higher yields than approaches in which only HNO_3 was used. The presence of highly stable organoarsenic compounds was suggested to cause this finding [38]. A similar observation was reported in a study on arsenic in certified mussel tissues. A normal pressure digestion with nitric acid underestimated arsenic concentrations, whereas satisfactory results were obtained with a mixture of HNO_3 , HClO_4 and H_2SO_4 [13].

Trace organic analysis involves somewhat more cumbersome methods than does inorganic analysis. In general, procedures have to be devised for sample pretreatment (homogenization and drying), extraction, clean-up and preconcentration and (depending on the compound and the technique for instrumental analysis) derivatization. The analyst has to consider the analytical problem as a whole and ensure that the methodologies used in the individual steps are well harmonized. The extraction efficiency should be scrutinized, considering such variables as the solvents, temperature and time. Sequential extractions should be carried out. As well as the extraction efficiency, the recoveries of standards added to the sample should be studied, in order to obtain insight into possible matrix effects. In the clean-up procedure, a compromise has to be found between the efficiency of separating the analyte from unwanted constituents and the loss of analyte. Clean-up procedures are prone to matrix interferences. One should test how rugged the procedures are for samples with very different characteristics. During the extraction and clean-up steps, manipulations such as the transfer of solutions and the evaporation of solvents have to be carried out, and losses of analyte must be controlled and reduced to a minimum. It has been recommended that one should keep the volumes above 2 ml until an internal standard has been added. The rate and temperature of evaporation should be controlled. The loss of analyte during solvent evaporation is most appropriately checked using standard solutions in which no substances are present, which may keep the analyte in solution. The final methods have to be specified in detail in the standard operating procedures and it is vital that the technicians adhere carefully to them [13,34].

It is stressed that materials with different origins and with different characteristics and interfering constituents should be employed in the research phase. Limitation to a single material bears the risk that the method cannot cope with the range of matrix characteristics and/or interferences encountered in practice. An example is provided by a study in which different procedures for the extraction of polychlorobiphenyls from fish tissues were compared [39]. Fish contain different types of lipids and distinction can be made between depot fats or free lipids, which consist of cholesterol esters and wax esters, and non-depot fats or bound lipids, which embody predominantly phospholipids. The PCBs in depot fats can be extracted with non-polar solvents when appropriate conditions are used. Depot fats are predominant for fatty tissues, so that acceptable results will be obtained for them using Soxhlet extraction with a non-polar solvent. In lean fish tissues, however, bound lipids constitute a considerable fraction. The extraction of PCBs from bound lipids requires saponification or Soxhlet extraction with a polar solvent. It was concluded that for the extraction of PCBs in fish tissues saponification was the method of choice, with Soxhlet extraction with polar solvents as a reasonable alternative [39].

It is important to incorporate into the methodology measures that enable a technician to assess whether the analytical procedure has been performed properly [40]. In chromatography one can use internal standards that behave like the substances under investigation. The use of isotopically labelled compounds constitutes an elegant approach for mass spectrometric techniques. Internal standards should be added at a very early stage. The technician can use the recovery of the internal standard to judge whether matrix interferences or gross errors have occurred. In ICP-AES, it is possible to determine the concentrations of a certain element routinely at two different wavelengths. With this approach, some matrix interferences may be detected. A number of analytical problems can be dis-

covered in time if a technician assesses the quality of the output on the basis of such measures and on the results from quality control samples in the form of blanks, reference materials and standard solutions (see below).

10.5.3. Validation of methodology

Method development will lead to a draft standard operating procedure. This draft SOP has to be subjected to a number of tests in order to validate it.

Validation has been described as “determining the suitability of methodology for providing useful analytical data”. A method is validated when the performance characteristics are adequate and when it has been established that the measurement process is in statistical control and produces accurate results [41].

More recently, the emphasis in validation has been put on the establishment and documentation of the specification of a test method. A set of performance characteristics to be assessed has been prescribed: precision, accuracy, limit of detection, limit of quantification, selectivity/specificity, range, linearity and ruggedness [20]. Unfortunately, this description of validation does not make explicit reference to statistical control. The performance characteristics are not meaningful if statistical control has not been achieved and demonstrated. In this context, a pronouncement of Eisenhart is often quoted: “Until a measurement operation has been “debugged” to the extent that it has attained a state of statistical control, it cannot be regarded in any logical sense as measuring anything at all” [42].

In the following, attention is focused on the ruggedness of methodology, statistical control and the assessment of accuracy.

In practice, small differences in operational and environmental conditions will occur. The sensitivity of the method with respect to realistic deviations from prescribed instructions, settings and conditions should be studied. Statistical approaches have been established to undertake this “ruggedness testing” in an efficient manner [43,44]. Ruggedness testing will provide an insight into the degree of long term variability that can be expected and how it can be controlled.

Analytical variability is inevitable. Problems with analytical methodology become manifest when fluctuations differ significantly from the normal, acceptable variations. It is essential that a laboratory perceives and masters the variation of its methodology.

Shewhart was the first to recognize the role of variability in manufacturing and measurement processes [45]. He stated the concept of “statistical control” [46] in this way:

A phenomenon will be said to be controlled when, through the use of past experience, we can predict, at least within limits, how the phenomenon may be expected to vary in the future. Here it is understood that prediction means that we can state, at least approximately, the probability that the observed phenomenon will fall within the given limits.

Shewhart developed statistical techniques and graphical means (control charts) to assess and monitor statistical control.

In many circumstances, statistical control can be assessed by measuring reference materials periodically and monitoring the results with control charts. Certified reference materials can be used, but their application is not recommended owing to their restricted

availability and costs [17]. A laboratory can prepare a batch of reference material itself. The material should be sufficient for a prolonged period of analysis. It has to be verified that the homogeneity and stability are adequate. The preparation of a large batch of material that is stable and homogeneous is not a simple undertaking. Low quality materials may even have a negative effect on the quality of the output of a laboratory [47]. It can be a good policy to obtain these materials from expert organizations, or to produce them in cooperation with other experienced laboratories.

Control charts provide a graphical means to monitor and interpret the data on reference materials or on other quality control samples. A set of 15 measurements on the reference material, obtained over a prolonged period of time, can be used to establish an estimate of the mean and standard deviation as a measure of the precision of the method [48]. This information is used to construct a Shewhart chart. It is assumed that the results are normally distributed. The properties of the normal distribution are used to predict the range in which the next measurements will fall and to establish criteria to allow one to assess whether the variations are acceptable. An example of a control chart is given in Fig. 10.3.

The concentrations are plotted on the vertical axis, the sequence number of the measurement on the horizontal axis. Horizontal lines are drawn at concentrations corresponding to the average concentration and to the average concentration ± 2 and 3 standard deviations. Normal statistics predict that 95.45% and 99.7% of the data will fall within the areas enclosed by the 2S and 3S limits. These boundaries are denoted as the lower and upper warning limits respectively (LWL and UWL in Fig. 10.3) and the lower and upper control limits (LCL and UCL in Fig. 10.3). The probability that a result will exceed the control limits is only about 0.3%. Therefore, it is assumed that an unacceptable fluctuation occurs if a transgression of a control limit takes place. Similar considerations lead to the conclusion that unacceptable fluctuations occur when a certain warning limit is ex-

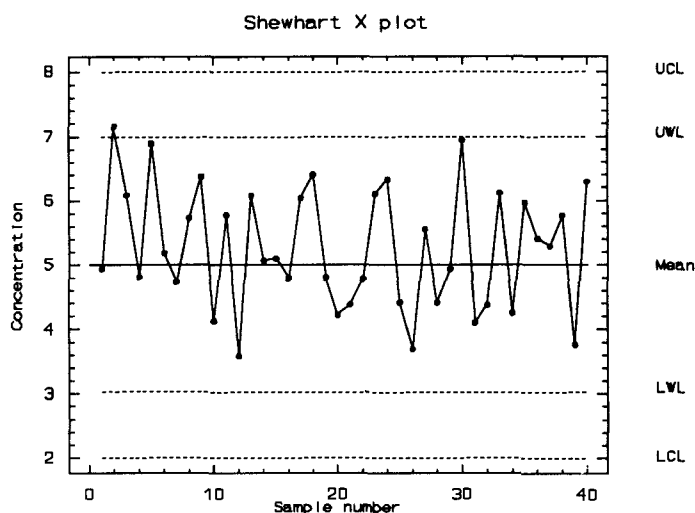


Fig. 10.3. An example of a Shewhart X chart.

ceeded twice in a row (probability 0.1%) or when 11 observations in a row fall to one side of the central line (probability 0.1%). The cusum chart forms an alternative to the Shewhart chart [19,49].

When reference materials are not available and cannot be prepared, statistical control may be monitored by plotting the difference of replicate measurements on a control chart (a Shewhart R chart [9]). It must be realized, however, that analytical variance depends, among other things, on the concentration level and on the matrix characteristics of the samples. Best results will be obtained when samples with a known history, comparable matrix characteristics and a limited range of analyte concentrations are employed.

Taylor describes five possible approaches to the assessment of accuracy: the use of spiked samples or surrogates; comparison of the test method with an independent method or with a method with known accuracy; the use of certified reference materials (CRMs); participation in laboratory performance studies [41]. Each approach has advantages and disadvantages. It is up to the professional judgement of the analyst to make optimal use of the options available. The use of spiked samples and of surrogates is not recommended. The other possibilities are briefly discussed.

A certified reference material (CRM) is defined as a reference material, one of more of whose property values are certified by a technically valid procedure, accompanied by, or traceable to, a certificate or other documentation which is issued by a certifying body [50]. By measuring CRMs, a laboratory obtains direct feedback about the performance of its methodology. A laboratory has to investigate its procedures if a significant difference between its results and the certified value is observed.

In the ideal case, a number of CRMs with a perfect matrix match, spanning the concentration range of interest, would be available. In reality, the choice is usually restricted to a limited number of CRMs. The analyst has to consider the matrix characteristics and concentration levels and find a reasonable compromise. CRMs fulfil a major role in environmental analysis. The expertise of experienced, highly qualified laboratories is used to establish the certified concentrations. All laboratories which measure these samples compare their results with this expert judgement. Through CRMs, accuracy is transferred among laboratories, contributing in this manner to measurement compatibility [51].

Not all CRMs are validated to the same standard. The user of CRMs should consider information on the preparation of the material, the results of homogeneity and stability tests, the number of different methods used to certify the material, the concentration levels, the matrix characteristics and the uncertainties in the stated values [20]. With CRMs, a major part of the analytical process is assessed and laboratories should make use of these materials wherever possible.

The assessment of accuracy by comparison with an independent method or with a method of known accuracy has perspectives for classes of chemicals, for example for trace metals. For many compounds, however, the possibilities seem limited at present owing to the limited availability of these methods. Usually, collaboration with other laboratories is necessary, although this may assess inter-laboratory comparability more than accuracy.

In line with Taylor, ISO Guide 25 and EN 45001 state that participation in "suitable" laboratory performance studies can provide evidence for accuracy. It is noted, however, that these inter-laboratory studies should have a proper method for the establishment of

assigned values [52]. Preferably, these values should be derived using results of an expert group of laboratories employing different and, where possible, independent methodologies, comparable to the way in which certification of reference materials takes place.

For a range of parameters and matrices, standard methods have been developed by the proper organizations (e.g. ISO, ASTM, CEN/CENELEC). It may be efficient to employ standard methods, which saves development time. The use of standard methods can also be prescribed by environmental legislation. Each laboratory should verify whether it fully masters a standard method before putting it into use. This implies a full validation in the laboratory along the lines described above.

10.5.4. Quality assessment

Laboratories should monitor the measurement process to ensure that the methodology remains under control. Periodically, parameters providing feedback about critical elements of the analytical process should be measured. In addition, arrangements should be made to check the output of a laboratory. These activities are denoted as quality assessment [53], although they are also referred to as quality control measures.

Quality assessment can be organized on three levels [54]. Quality assessment on the first level has the objective of verifying the proper functioning of the analytical process and identifying problems at an early stage. Quality assessment on the second and third levels provides feedback about the laboratory performance.

The first level entails the technicians and analysts carrying out sampling and analyses. These personnel should plan their work well. Protocols should be adhered to. The protocols should contain measurements to verify control of the analytical process. Options include checks on:

- contamination by determining procedural blanks;
- drift in calibration by measuring standard solutions in measurement series;
- instrument performance by periodically assessing sensitivity;
- statistical control with reference materials or with replicate analyses as described earlier;
- accuracy with CRMs.

Control charts provide a convenient method to monitor the results of these measurements. The number and frequency of checks is to be chosen taking into account the stability of the measurement system, the matrix characteristics of the samples, the concentration levels of the analytes and the sample throughput. The WELAC/EURACHEM guide states that 5% of sample throughput should consist of quality control samples for routine analysis and 20% up to 50% for more complex procedures. A full system validation is prescribed for analyses which are performed infrequently [20].

The technicians should maintain the control charts and critically evaluate the results of quality control measurements (e.g. replicates, reference materials, blanks). Relevant observations and details should be written down. The technician should take action if irregularities are observed. The allocation of this responsibility to the technicians has the positive side-effect that they become more involved with their work [50]. The manager of a laboratory sector should periodically evaluate control charts and other quality assessment data and discuss the results with the personnel.

The second level of quality assessment invokes "blind samples" distributed by the quality assurance unit. Blind samples are quality control samples such as reference materials or replicates. The nature of these samples should not be disclosed to the technicians.

The third level of quality assessment entails participation in laboratory performance studies. These studies are organized by an independent organization. Preferably, these samples should be treated as blind samples, that is, they should be included in the routine measurement series and not receive special attention. Participation in laboratory performance studies is strongly recommended (e.g. in ISO Guide 25, EN 45001 and the WELAC/EURACHEM guide).

It is to be noted that the results obtained for blind samples and in laboratory performance studies should also be communicated to the technicians.

10.6. INTER-LABORATORY STUDIES AND THEIR FEEDBACK

Inter-laboratory studies is a generic term for research that involves the analysis by a number of laboratories of identical samples for a particular purpose. Table 10.1 presents an overview of the different types of inter-laboratory studies, together with their objectives [55]. The terminology used follows the recommendations put forward by IUPAC [56].

Method performance studies may be performed to detect the weaknesses of methods and to remedy them, to study the robustness of a method and/or to validate methodology and to determine its performance characteristics. The Association of Official Analytical Chemists (AOAC) has done much in this field. Various standards and regulations have been published for method performance studies, which discuss the organization, statistical design, execution and evaluation of inter-laboratory studies [57,58].

For the validation of methods, adequate reference material is of special importance. In relation to this, inter-laboratory studies are organized to determine the reference values of components or features of a reference material (material certification study).

The certification of materials is a highly specialized activity, which is supervised by a laboratory or institute whose expertise is generally recognized (e.g. the EC Measurements and Testing Programme (formerly BCR), US-NIST). Expert laboratories are invited to participate in this type of inter-laboratory study. Attempts are made to include different

TABLE 10.1

Objective	Type of inter-laboratory study
Making available well-validated methods	Method performance study
Making available certified reference materials for the evaluation of systematic error	Material certification study
Testing the performance level of a laboratory	Laboratory performance study
The participation in inter-laboratory studies by a laboratory to assess accuracy	Laboratory performance study
Quality improvement projects	Laboratory performance study

analytical methods, which are as independent of one another as possible, in order to avoid method-related systematic errors [59].

The statistical design of certification or consensus studies has not been elaborated in detail in the form of a standard.

The quality improvement programmes employ laboratory performance studies as tools in a learning programme. The laboratories collectively approach a particular analysis problem under the leadership of a project team. The analytical procedure is followed step by step, starting with the calibration. A method is not prescribed. The EC Bureau of Reference (BCR) has developed this method with great success [60,61].

Laboratory performance studies may be realized in various ways. In a number of cases there is a fixed scheme according to which the laboratories are offered samples for analysis. The tests are of a routine nature and constitute an integrated part of the quality control programme of a laboratory. Laboratory performance studies may also be incidental exercises, for example to assess the comparability of laboratories. Many laboratory performance study schemes have been developed as a response to the clients' wish to gain more insight into, and a greater control of, the quality of analyses.

There are still only a small number of regulations for laboratory performance studies. Many activities have been developed internationally in order to reach a protocol (AOAC/ISO/IUPAC) [48]. The obstacles are mainly in the field of statistics.

Laboratory performance studies provide feedback on three levels:

- (1) The individual laboratories obtain information on their performance. Each laboratory should evaluate the findings of the study and look into its methodology if its results exhibit unacceptable deviations.
- (2) Third parties, as accreditation bodies or managers of monitoring programmes, obtain information on the performance of individual laboratories. This information can lead to inquiries about poor performance or to more radical measures.
- (3) The whole sector is informed on the state of the art with respect to analytical proficiency. Poor overall performance points to the need for harmonization of methodology, validation of harmonized methods via method performance inter-laboratory studies, and/or the preparation of (certified) reference materials. Poor overall performance may also point to inadequate design of, for example, monitoring programmes owing to insufficient specification of the analytical methodology.

The types of feedback which can be obtained are illustrated with a number of examples. In Fig. 10.4, the results of a laboratory performance study on organic carbon in sediments [62] are presented in the form of a two-sample plot. For each laboratory the result for sample A is plotted on the *X*-axis and the result for sample B is plotted on the *Y*-axis. When certain conditions are met, the scatter exhibits a circular pattern if only random errors are present. Alignment points to systematic errors [63].

The plot given in Fig. 10.4 suggests strongly that systematic errors occur. Inspection of the methodology revealed that different techniques were used and that the results from laboratories using the same technique formed clusters. It was concluded that the patterns resulted from systematic differences between the methods employed [64].

In the Netherlands, organic carbon and the mineral fraction smaller than 2 μm are used in the assessment of the contamination of dredged material. The concentrations of metals are corrected for the composition of the sediment according to the general formula

$$M_{\text{corrected}} = M_{\text{measured}} \times (a + 25b + 10c)/(a + Lb + Hc)$$

The constants a , b and c differ for each metal. L represents the mineral fraction smaller than $2 \mu\text{m}$ (in %), H represents organic carbon (in %). For organic contaminants, the correction is only based on organic carbon ($a = b = 0$). The corrected contaminant concentrations are compared with environmental quality criteria. It is clear that the method for the determination of organic carbon must be specified in environmental legislation, to eliminate the discrepancies caused by the application of methodologies which have systematic differences.

Figures 10.5 and 10.6 present examples of the results of laboratory performance studies on trace metals and trace organics in dredged material. Both plots point to systematic differences between laboratories. The degree of incomparability is high for trace organics.

For trace metals, no relationship between methodology and systematic deviations could be found. This can be compared with the findings of van Raaphorst [13] and Berman [47], who both came to the conclusion that different methodologies in trace metal determinations give comparable results when the techniques are applied correctly. It appears that laboratories do not pay enough attention to quality assurance.

The analysis of organic compounds in sediments and soils is fraught with problems. Well validated, standardized methods are in the stage of development (adequate methodology, however, is present). In addition, certified reference materials are sparse.

The results of inter-laboratory studies indicate that the methodology is not sufficiently validated, in particularly with respect to the assessment of accuracy. The dominance of systematic errors in the plots suggests that measures to improve and transfer accuracy may be very effective. The results underline the importance of certified reference materials.

The determination of contaminants, such as chlorobiphenyls and polycyclic aromatic hydrocarbons, in dredged material is important for Dutch environmental management.

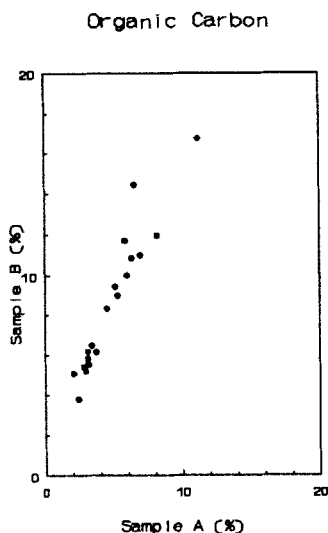


Fig. 10.4. A two-sample plot for organic carbon in sediments.

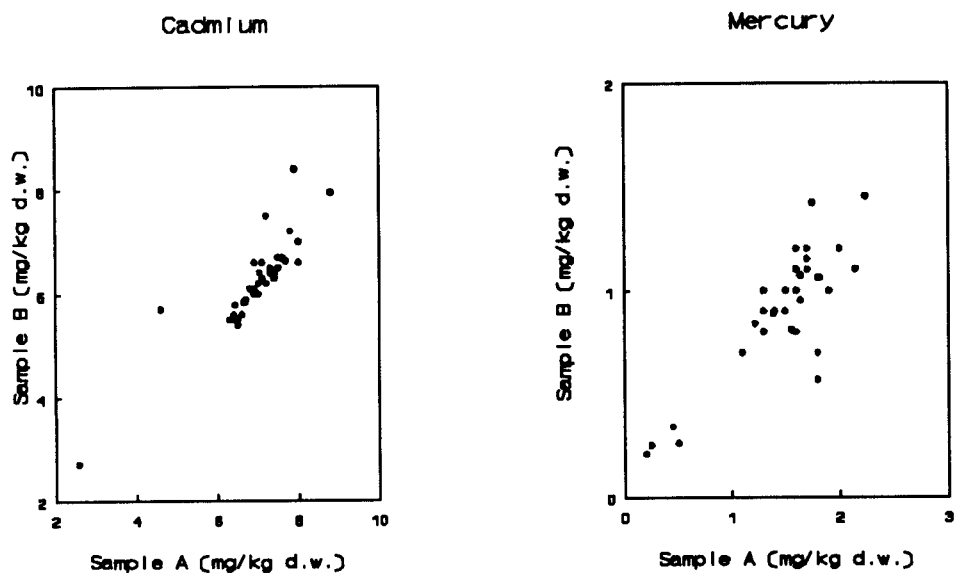


Fig. 10.5. Results of a laboratory performance study on Cd and Hg in fresh water sediments [62] depicted as two sample plots.

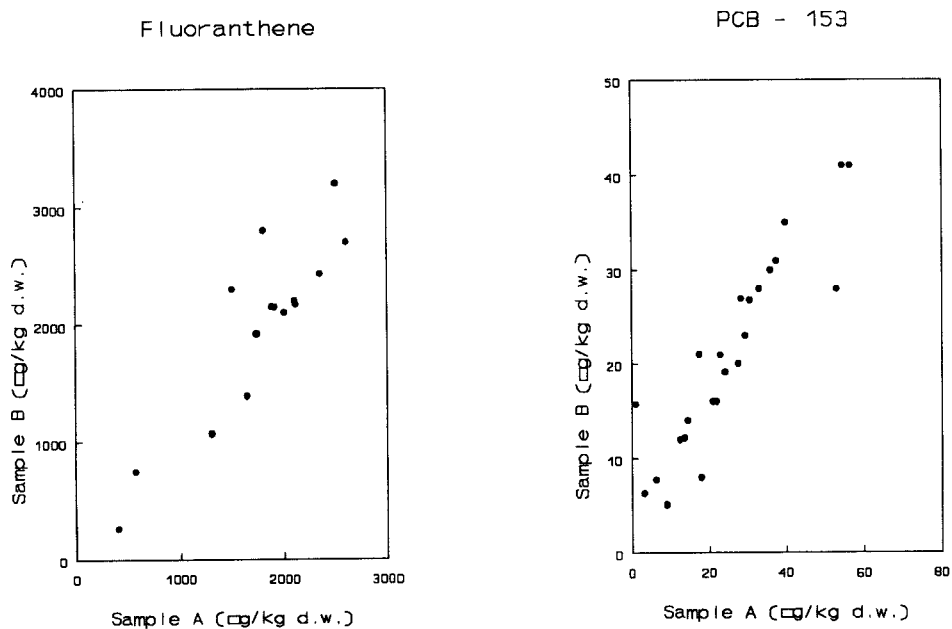


Fig. 10.6. Results of a laboratory performance studies on fluoranthene [70] and PCB-153 [71] in fresh water sediments depicted as two sample plots.

The disquieting results of the inter-laboratory studies have provided an impetus for the Dutch Government to improve analytical capabilities. To this end, the development of well validated standard methods and of reference materials has been given high priority [65]. Inter-laboratory studies conducted among laboratories engaged in monitoring the North Sea also revealed analytical difficulties [66–68]. Quality improvement programmes have been established for the determination of chlorobiphenyl and polycyclic aromatic hydrocarbons in marine media [31,32,35] and will be expanded in the context of an project of the European Community [69].

The assessment of a laboratory on the basis of a laboratory performance study is not as straightforward as it may seem [55]. Not all inter-laboratory studies are carried out to the same standard. A laboratory must make its own evaluation of whether the organization and realization of the inter-laboratory study justifies the drawing of conclusions about its performance. Factors which must be taken into consideration are:

- the number of participants and their experience with the methodology tested;
- the origin and representative nature (e.g. character) of testing samples;
- the number and types of methods which have been used in the inter-laboratory study;
- the organization of the study (e.g. the statistical model, the number of replicates, the parameters to be measured, the manner of execution);
- the arguments used in the evaluation of the participants' position. The spread in the laboratory data and the systematic differences observed in Figs. 10.4, 10.5 and 10.6 highlight the need for thorough procedures for the establishment of assigned values, as has been discussed earlier.

The statistical handling of the results by the organizers of the inter-laboratory studies must be viewed as an aid to self-evaluation. In context, attention must be paid to the results for the individual parameters for each sample and to the results for all parameters in relation to the nature of the samples. Furthermore, the laboratory's own performance must be considered in the light of the results of the quality control measurements and the inter-laboratory studies in which it has previously participated [55].

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Chapter 11

Certified reference materials for the quality control of measurements in environmental monitoring

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11.1. PARTICULAR ASPECTS OF ENVIRONMENTAL ANALYSIS

The United Nations' Summit on the protection of the environment held in Rio de Janeiro (Brazil) in June 1992 was a confirmation that environmental protection has gained worldwide interest and is now a priority for several countries. The public concern and, consequently, the economic and political impact of environmental protection, has led to the development of several projects. In some cases regional, national or international regulations (e.g. E.C. Directives) or international conventions such as the Paris and Oslo convention for the North Sea, or the Barcelona convention for the Mediterranean Sea, re-

quire monitoring programmes and sometimes introduce maximum allowed concentrations for certain contaminants. These monitoring programmes have one common prerequisite: that measurements of various parameters are necessary to evaluate the situation and follow its evolution. The results of the determinations performed provide a basis for the decisions taken by the authorities and for possible actions. Their effect is again evaluated using measurements which are conducted over long periods of time. Trends, and even the rate of decontamination processes may be established, as a result of which actions will be modified. The economic and legal impact of the decisions (closing of factories, restraints in the workplace, waste management etc.) and the human effect (unemployment or the displacement of populations as in Seveso in Italy or Times Beach in the United States) can be enormous. Therefore, the analyses have to be as reliable as possible.

11.2. RELIABILITY OF DATA

Reliable data are the result of a chain of actions which starts with the proper definition of the problem to be solved. This should lead to a clear identification of the parameter(s) concerned. It includes the target samples to be selected, the sampling strategy and the proper sampling technique to be applied. When adequate samples have been analysed, the results should be reported in such a manner that those who have to draw the conclusions and to take action, can do so with all possible assurance.

Between the sampling and the reporting of the data lies the difficult work of the analyst in the laboratory. This closes the circle of the total quality control procedure as defined by Cofino et al. [1]. Proper determinations of chemical parameters mean that precise (repeatable and reproducible) and accurate results are delivered. To achieve the best possible precision within the state of the art, the analysts have to work in the most favourable environment. The laboratory has to be organized according to a quality assurance program. This includes infrastructure, personnel qualification and motivation, work load, maintenance of apparatus, proper chemicals, adequate management, etc. A comprehensive quality assurance manual should be available.

Only methods validated in detail should be used. These methods should be under statistical control [1]. The survey of the method can be followed by control charts, as described in Section 11.5. It has been demonstrated that a good quality assurance programme is essential to the accuracy and precision of analytical results [2]. When all possible measures have been applied in the laboratory to improve the precision, the analyst can concentrate on obtaining accuracy.

11.3. THE NEED FOR ACCURACY

The International Organization for Standardization (ISO) defines accuracy as: "the closeness of the agreement between the result of a measurement and the (conventional) true value of the quantity to be measured".

As said before, an enormous number of analyses are being performed for the purpose of monitoring the environment. Together with the diversity, the number of materials to be

determined, and the wide range of concentrations, one has to consider the complexity of the matrices to be studied. Current analytical techniques with powerful instruments, which are often to a large extent automated (including the interpretation of the data), have made it possible to routinely determine concentrations of 10^{-12} g/g or quantities of 10^{-12} g. This achievement in sensitivity has been obtained along with the high throughputs of analyses which are normal nowadays. However, these very high sensitivities do not necessarily improve accuracy [3]. Too many scientists have stated that a good reproducibility over time is sufficient to allow one to follow trends and demonstrate the effects of actions carried out by the authorities to improve the quality of the environment. Such a statement overlooks the application of results for modelling, and the development of eco-toxicological and ecological theory, etc., and ignores improvements in equipment and methodology. There are still too many analysts who confuse accuracy with precision: the within-laboratory repeatability and reproducibility are both essential for the monitoring of the environment but are not sufficient. Without accuracy, results from different groups in different parts of the world are not comparable, although they should be.

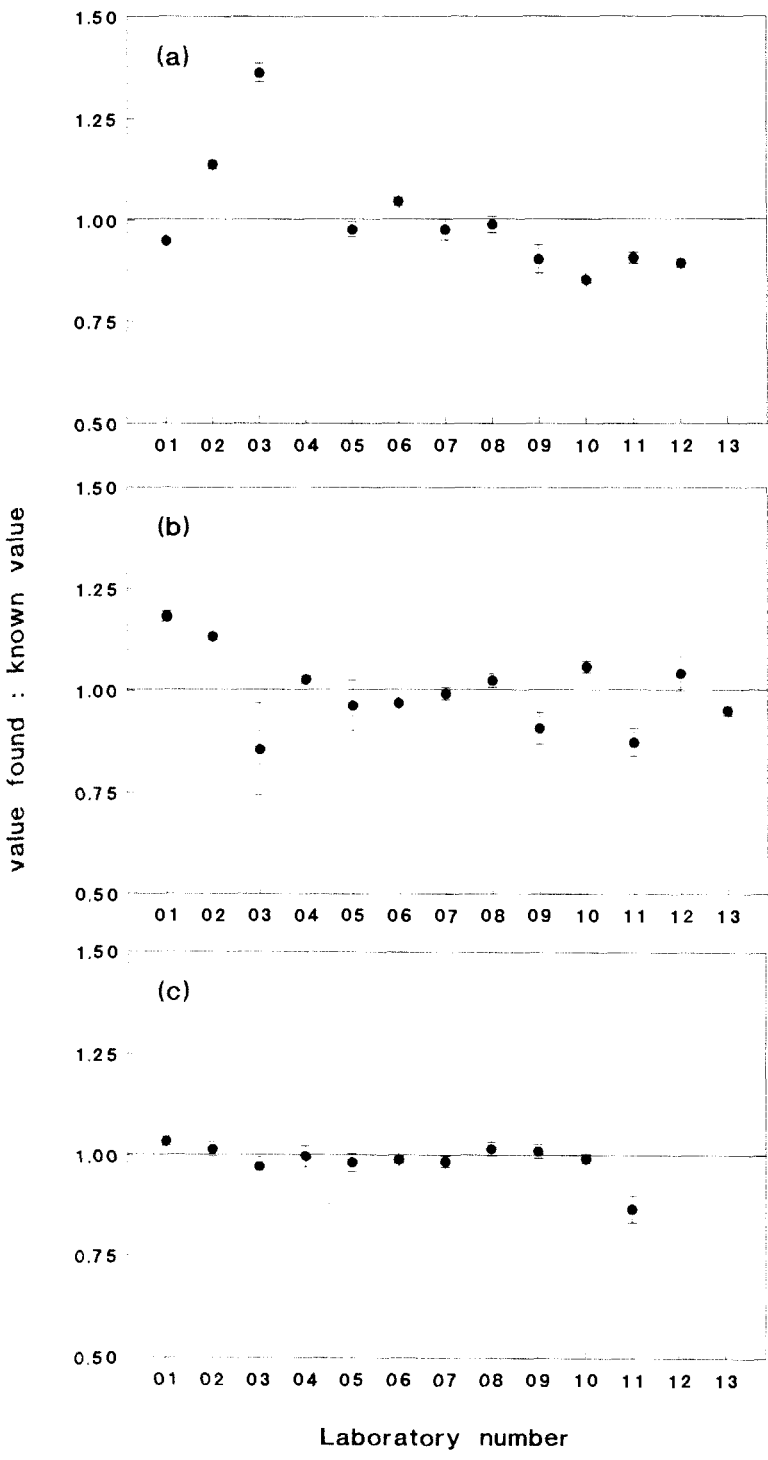
Accurate measurements are expensive but bad measurements cost much more. An example illustrating the usual accuracies achieved by laboratories of good reputation is given in Fig. 11.1. This example dealt with measurements of air in the workplace. Tenax sorption tubes were charged with vapours (approx $1.0 \mu\text{g}$) of benzene, toluene and *m*-xylene and distributed for analysis to several well trained and selected laboratories. In the first interlaboratory study, the results differed from the target value by more than the legally allowed limit (10%). Only after three exercises were the participants able to meet the required accuracy. Numerous other examples can be given [4–10]. To achieve and prove the required accuracy, several conditions have to be fulfilled.

11.4. HOW TO ACHIEVE ACCURACY

Concern about the lack of reliability of results has grown over the last decade. Good Laboratory Practice (GLP) rules were first established for toxicological testing laboratories by the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) in the United States, as well as the Organization for Economic Co-operation and Development (OECD). For other fields of measurements, the quality criteria and requirements are given in the international standards issued by ISO and the European Committee for Standardization (CEN), e.g. the ISO 9000 [11] and EN 45000 [12] series of norms. They describe the requirements for laboratory organization, competence of management and personnel, the laboratory quality manual, the need for proper calibration of instrumentation, the use of reference materials, and the participation in interlaboratory studies.

11.4.1. Comparison with a different method

Each method has its own sources of error to which those of the analyst applying it have to be added. For spectrometric methods, it can be the digestion of the matrix. Although this is not a problem in instrumental neutron activation analysis (INAA), INAA may have



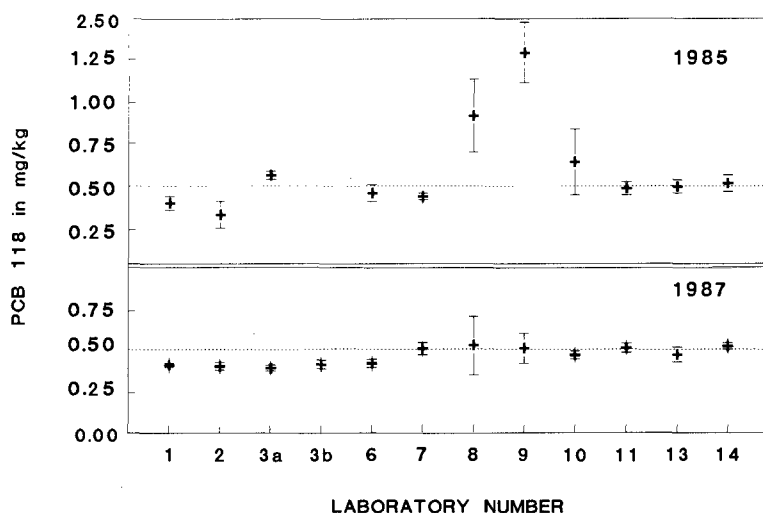


Fig. 11.2. Improvement in the between-laboratory agreement for the determination of CB 118 in two similar fish oils: herring in 1985 and mackerel in 1987 [39].

errors due to shielding, insufficient separation of gamma-peaks, etc. An independent method, e.g. inductively coupled plasma optical emission spectrometry (ICP-OES) instead of INAA, can be used to verify the results of routine analysis. If both methods are in good agreement, it is likely that the results obtained by the routine method will not be affected by a systematic bias. The conclusion is most valid when both methods differ widely. If the methods used for this verification have similarities, such as the pretreatment, a comparison may overlook systematic errors due to the common step. If the technician is not sufficiently experienced with the comparison method it may even create additional errors.

11.4.2. Comparison with other laboratories

The laboratories participating in interlaboratory studies often observe that their results are scattered. Figure 11.2 illustrates such a situation for the determination of polychlorinated biphenyls (PCBs) in fish oils. Figure 11.3 shows the achievements of a group of laboratories working on the determination of polychlorodibenzo-*p*-dioxins (PCDDs) and polychlorodibenzo furans (PCDFs). They used a so-called step-by-step approach consisting of several interlaboratory studies. In each exercise, they had to analyse adapted mate

Fig. 11.1. Results of three intercomparisons for toluene trapped on Tenax (mean value \pm 1SD). Known value = value calculated from the preparation data of the tubes. (a), (b), (c): first, second, third round. After each round the results were examined and discussed in a meeting with all participants [38].

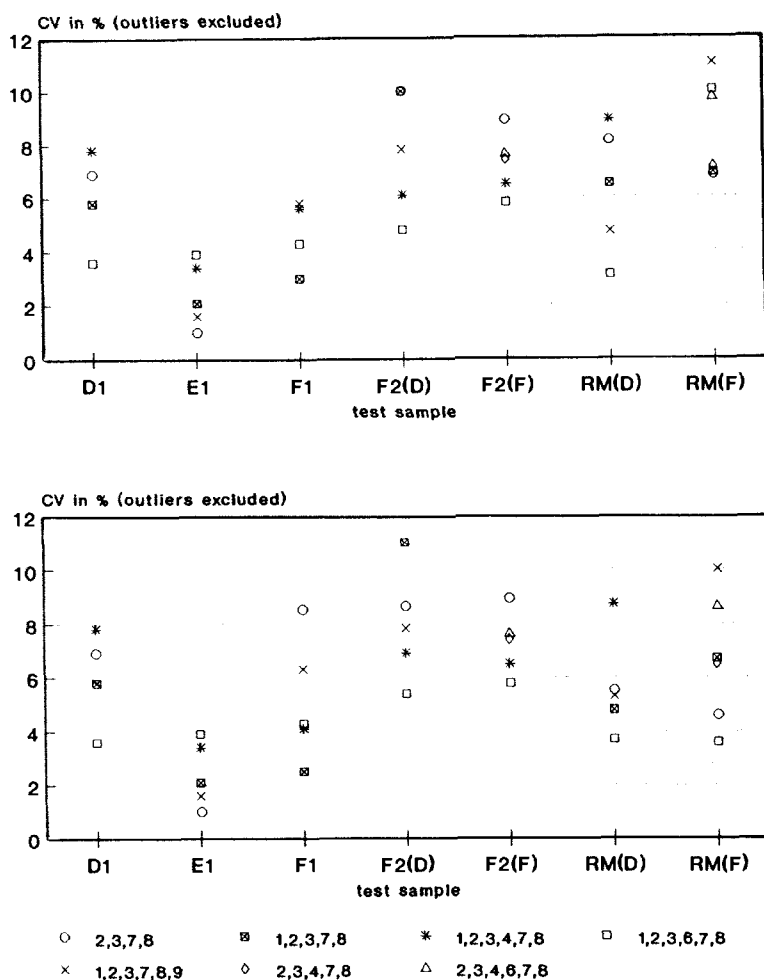


Fig. 11.3. Interlaboratory studies on the determination of PCDDs and PCDFs in fly ash (upper graph for all participants, lower graph for constant participants). The group followed the scheme presented in Fig. 11.4. The data presented in the upper and lower graphs are the coefficient of variation of the mean of means after exclusion of outliers on a technical basis following the discussion between the participants. D1, 1st solution PCDD; E1, 2nd solution PCDD (with properly controlled and common calibrants); F1, PCDD and PCDF in clean fly ash extract; F2(D), PCDDs in raw fly ash extract; F2(F), PCDFs in raw fly ash extract; RM(D), PCDDs in CRM 429; RM(F), PCDFs in CRM 429 (fly ash extract).

rials to validate each step of their analytical procedure. Figure 11.4 details the scheme followed in this latter study. The participation in such interlaboratory studies, combined with a critical discussion of the results, is very useful for obtaining a high level of accuracy. Experience indicates that once a good level has been achieved, continued participation is necessary to maintain the good measurement quality [13]. It can be expected that in the

near future the opportunity to participate in interlaboratory studies will increase, as such participation is recommended by accreditation bodies following the prescription of the ISO 25 [14] and EN 45000 series of norms [12]. Several bodies are contemplating the organization of interlaboratory studies, with or without proficiency objectives. International, as well as national, organizations are normalizing the rules for the organization of such studies in order to guarantee a maximum benefit for the participants [15]. However, there is not always a proficiency scheme in the field of interest of the laboratory.

11.4.3. Certified reference materials: definitions (ISO) [16]

Reference material (RM): a material or substance one or more properties of which are sufficiently well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

Certified reference material (CRM): a reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body.

The use of CRMs is the easiest way to achieve accuracy. Certified reference materials of good suppliers link the user's results to those of the international scientific community. Additionally, they enable the user to verify his performance at any desired moment.

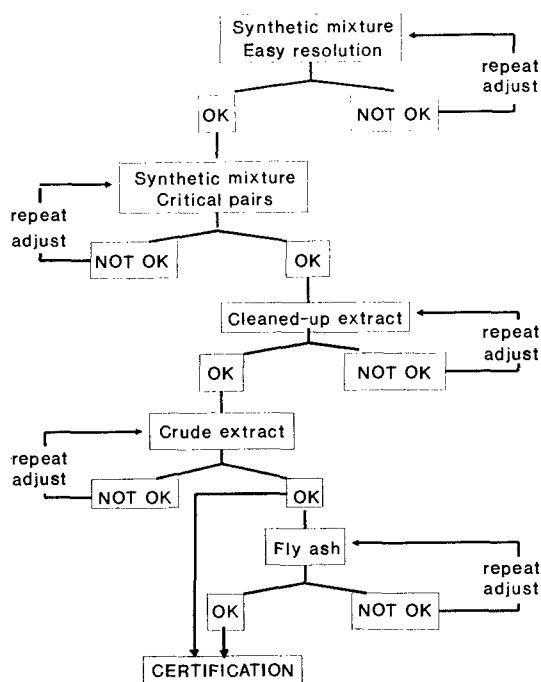


Fig. 11.4. Improvement scheme for the determination of PCDDs and PCDFs in fly ash [40].

11.5. USE OF CRMS

11.5.1. Calibration and traceability

If one wishes to investigate the accuracy of an analytical procedure, it is important to study each step of the process, from weighing the sample to the final determination. No measurement can be valid if all instruments used in the course of the analytical procedure are not properly calibrated (e.g. calibration of balances, volumes of glassware and pipettes, instruments). Modern methods of final determination are based on the measurement of a signal which must be correlated with the concentration of the substance of interest in the unknown sample. This can be a chemical element, a chemical form of an element (e.g. metal speciation) or a chemical compound in organic analysis. The correlation is established by means of a calibration curve. The solutions used to construct the calibration curve must be prepared with chemicals of an appropriate purity and verified stoichiometry [17]. The matrix effects should be estimated and the calibration solutions should be "matrix matched". If all steps of the procedure are fully under control and all uncertainties have been estimated, the measurements of the analytical sample (digest, solution, clean-extract, etc.) can be considered to be traceable to defined chemical species.

In fact, in chemical analysis the measurement process contains steps where the sample is physically destroyed (e.g. acid digestions, fusions, calcinations) or the analyte is extracted from the matrix. To ensure accuracy, it is necessary to demonstrate that no losses or contaminations occurred in the course of the sample treatment. The laboratory can verify the analytical procedure with a reference material having a matrix similar to the unknown sample and certified in a reliable manner. Any disagreement between the certified value and the value determined by the laboratory indicates the presence of an error in the analytical procedure. When there is no disagreement between the found and the certified value, the laboratory can conclude that the method has been properly applied with this particular sample. The CRM plays a role similar to a metrology transfer standard and will allow the laboratory to link the measurement process to an internationally recognized reference [18].

Unfortunately, as will be discussed in more detail later, the total similarity of CRM and real samples cannot be always assured for reasons of stability or homogeneity. Therefore, some uncertainty regarding the accuracy of determination of the real samples will remain. This is a difference from metrological measurements, where there is usually a total similarity of the transfer standards and real samples.

11.5.2. Accuracy of a method

When developing a new analytical method or apparatus and after having evaluated all its critical points, the analyst has to prove the accuracy of the measurements. Usually the results are compared with those obtained with a classical method. This implies that this classical method is fully under control in that laboratory. However, some critical factors, such as the influence of laboratory contamination, cannot be solved in that laboratory it-

self and would need to be investigated in another laboratory. It is in fact much easier to use a CRM to evaluate the accuracy if a suitable CRM exists.

CRMs may also be used to test a standardized method when it is applied for the first time in the laboratory or when a new technician is applying it. The use of a standardized method is not a guarantee of accuracy [19] and does not protect against mistakes and criticism. Sometimes very qualified laboratories have difficulties in putting written standards into practice. These standard procedures are often elaborated by very senior analysts who may forget that they will be applied by less qualified people in charge of routine measurements. It also has to be stressed here that standard procedures may block the improvement of the methodology applied in the laboratories. In fact, by prescribing a procedure, one may prevent the use of recently developed methods which may be more sensitive and less subject to systematic errors. Unless the standard procedure is essential to define the parameter to be measured (see item (d) in Section 11.6), it would be more effective to authorize the use of any method whose accuracy has been proven by the use of a suitable CRM.

11.5.3. Evaluation of working standards and statistical control schemes

When a laboratory works at a constant level of high quality, fluctuations in the results are small [20]. As soon as a method is under control in a laboratory, control charts should be made to detect possible drifts. A control chart is a graphical representation of the results obtained for the measurement of a reference sample or working standard. This should be a material similar to the unknown samples, with a proven homogeneity and a good stability. Figure 11.5 shows examples of control charts. For a more rapid detection of trends (introduction of systematic errors) or drifts, cusum charts (cumulative sum) can be used [4,21,22].

Working standards, also called laboratory reference materials (LRM), may additionally be compared with similar CRMs to try to maintain the accuracy of the method. It must be noted that this traceability to CRMs is not easy to achieve in one single laboratory and that it would be preferable to have the LRM analysed by different good laboratories, applying different methods, as is often done by certification bodies. The need for a reference value, X_{ref} in Fig. 11.6, requires the use of good LRMs which are traceable to CRMs. The homogeneity and stability of LRMs can be verified in a similar manner as for CRMs (see Section 11.6).

The organizations producing CRMs would not normally become manufacturers of large quantities of LRMs. This is a task for industry as in the case of clinical chemistry [18], or should be done by each laboratory itself, possibly in collaboration with others.

11.5.4. Other uses of CRMs

CRMs can also serve the very useful purpose of demonstrating the equivalence of methods. This enables laboratories to follow the development of new analytical instrumentation. The analyst can compare the performance of his method with those from other laboratories without the necessity for intercomparisons. Such use is essential when har-

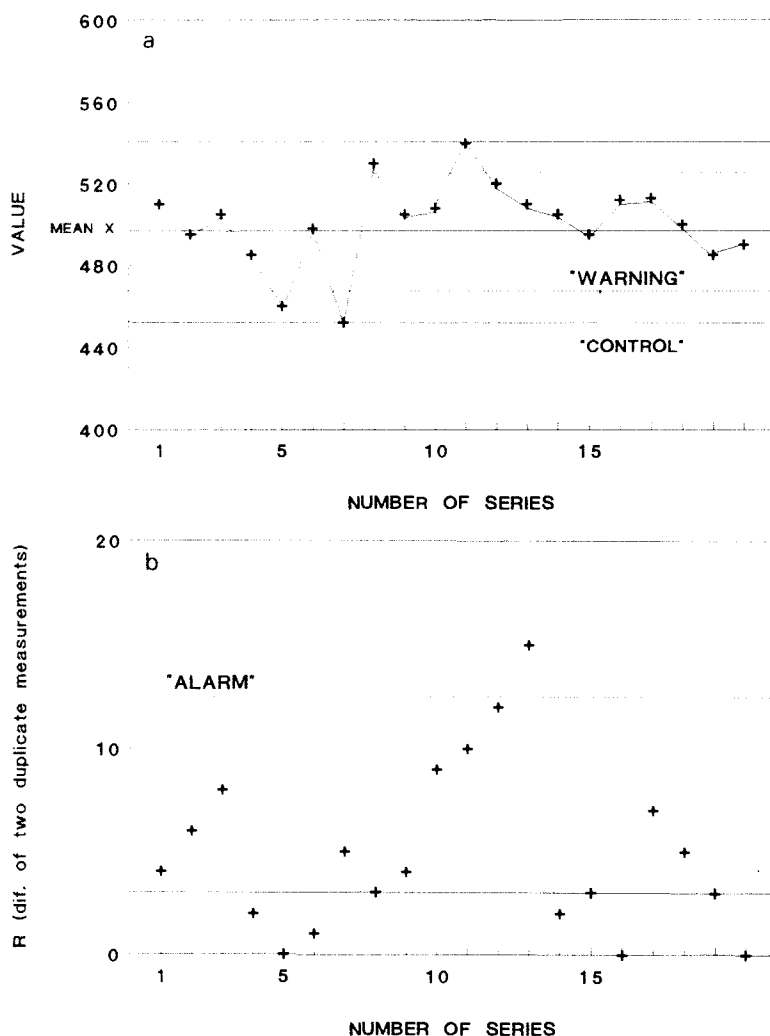


Fig. 11.5. Examples of \bar{X} and R charts (arbitrary units). \bar{X} is the value obtained at each occasion of analysis. R is the difference between two duplicate determinations. Warning and alarm lines correspond to a risk of 5 and 1%, respectively, that the result does not belong to the whole population of results.

monization of measurements is necessary at an international level (e.g. preparation of European Community Directives).

Some methods, e.g. optical emission spectrometry, wavelength dispersive X-ray fluorescence, are calibrated with reference materials of a similar, fully characterized matrix (e.g. metal alloys). For such methods, accuracy can only be achieved when certified reference materials are used for the calibration.

This is not developed here as it is not specific to environmental monitoring.

11.6. REQUIREMENTS FOR CERTIFIED REFERENCE MATERIALS

CRM's can be:

- (a) pure substances or solutions to be used for calibration and/or identification;
- (b) matrix reference materials which, as far as possible, represent the matrix being analysed by the user and which have a certified content (such materials are mainly to be used for the verification of a measurement process);
- (c) materials of a known matrix composition for the calibration of a certain type of measuring instrument, e.g. spark source emission spectrometry, X-ray fluorescence (XRF), and those techniques which require a calibration with a material similar to the matrix analysed;
- (d) methodologically defined reference materials for parameters such as: leachable or aqua-regia-soluble fractions of trace elements from soils, ashes and slags; bio-availability of a certain element, chloroform-extractable pesticides, etc.). The certified value is defined by the applied method following a very strict analytical protocol.

Attention will mainly be focused on matrix materials (category (b)).

CRMs have to fulfil many requirements. In order to assume properly their role in helping laboratories to achieve accuracy, they should be as similar as possible to the unknown sample to be analysed. It is not possible to fulfil all requirements. Sometimes compromises are necessary; highly unstable compounds or matrices cannot be certified.

11.6.1. Selection of candidate CRMs

To verify the results of the analysis of a certain matrix, one should select a CRM which is similar to the unknown sample or which is at least as difficult. In most cases this means similarity of:

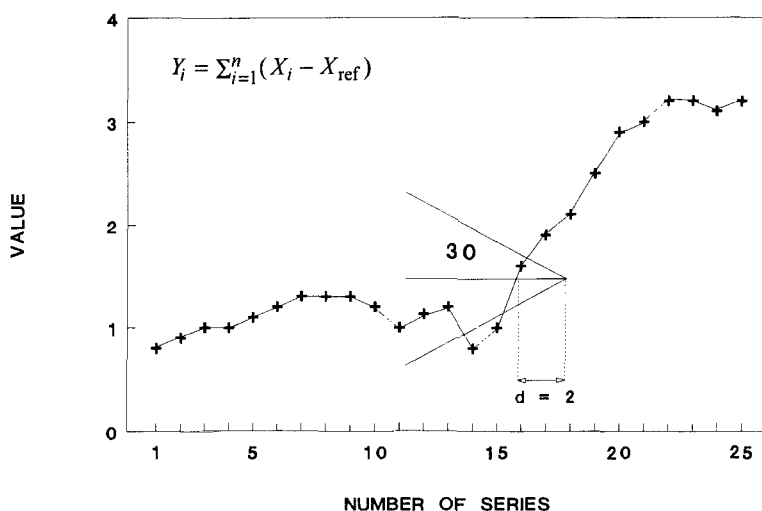


Fig. 11.6. Cusum chart. The sum of the differences between the found result and a reference value are plotted in time. They allow the detection of drifts and trends.

- the matrix composition;
- the contents of the analytes;
- how these analytes are bound;
- the fingerprint pattern of possible interferences;
- the physical status of the material.

In preparing a candidate CRM, these items should be taken into full consideration. In particular, for the speciation of, e.g. trace elements, or for the determination of organic compounds bound to the matrix, artificially spiked materials are not representative of real samples [23–25].

However, for practical reasons this similarity cannot always be respected. The material has to be homogeneous and stable in order to ensure that the samples delivered to the laboratories are identical. Therefore, compromises have to be made, and the preparation of the material has to be adapted.

11.6.2. Preparation of the material

The material has to be collected in a quantity sufficient to assure an adequate stock. CRMs should be available over several years to fulfil properly their task in the quality control and quality assurance system set up by the laboratories. Therefore, the quantity to be collected may be as much as 100 kg of solid material (e.g. soils or sediments) or several cubic metres (e.g. water CRMs). The producer needs to be properly equipped to treat such large amounts of material. The collected material has to be stabilized. This is one of the most sensitive and difficult steps in the work. The stabilization has to be adapted to each particular case and should be studied in detail before processing in order to respect the integrity of the material as much as possible. Usually the materials are dried to avoid chemical or microbiological changes. This may be achieved by heat or by freeze-drying, depending on the volatility of the analytes and matrix components. Some materials can be sterilized by gamma-irradiation (^{60}Co source). In reality this treatment is only possible for materials to be certified for inorganic parameters as many organic compounds are destroyed by gamma-irradiation [26]. Simple freezing of the material is also possible but the resulting material can only be used once as refreezing may not lead to a homogeneous material.

The material must also be homogenized and stored in adequate vials. For gases and liquids, the achievement of homogeneity is not the most difficult problem; their stability, however, causes great concern. Solid materials are difficult to homogenize. When the particle size is lower than 125 μm and when the particle size distribution of the material is sufficiently narrow, it has been shown that the homogeneity is sufficient even for sample intakes of less than 50 mg [27]. This can be achieved by a proper grinding procedure and a thorough homogenization before and during the filling procedure. Unfortunately, the low particle size has its drawbacks as it leads to materials which are usually easier to analyse than real samples owing to better extractability of analytes or easier acid or solvent attack on the matrix because of the large contact surface. Static electricity may cause subsampling difficulties for some materials with very low particle sizes and low water content [28].

11.6.3. Homogeneity and stability

A test portion of CRM in chemistry can be used only once when taken out of its storage vial. Therefore, the quantity of material in the bottle or ampoule should be sufficient to allow several determinations (20–50 g). A verification of the homogeneity has to be performed to ensure that, within a bottle or ampoule and from one vial to another, the content is the same (within- and between vial-homogeneity). The producer of the CRM should verify the minimum sample size for which the homogeneity is sufficient, i.e. for which he can guarantee that the uncertainty caused by inhomogeneity does not exceed the uncertainty of the certified values [29,30]. Usually, matrix materials segregate during transport. Therefore, in addition to the homogeneity data, the producer of the CRM should supply the user with sufficient information on how to rehomogenize the sample.

The producer of CRMs also should guarantee that the characteristics of the material and the certified parameters remain unchanged over longer periods. He should study the long-term stability beforehand to assess the bench life and possible damage during transport. The stability can be estimated by evaluating the behaviour of the material under accelerated aging conditions [26]. Stability checks may continue at regular intervals after certification during the entire availability of the CRM.

11.6.4. Certification

The certified value should be an accurate estimate of the true value with a reliable estimate of the uncertainty. The ISO Guide 35-1985 [31] gives several technically valid approaches for certifying a reference material. Depending on the type of RM and parameter to be certified, there may be some differences in the approach applied. Only certification of matrix materials is discussed here.

One method of certifying a parameter in a RM consists in using a so-called definitive method, e.g. isotope dilution mass spectrometry (IDMS) in inorganic trace analysis, in one single laboratory [31,32]. However, this laboratory may have a bias and therefore the certified value may be wrong. This method of using the results from one method in a single laboratory does not give a fair estimate of the uncertainty as achievable by other, more classical methods. In addition, such “definitive methods” hardly exist in organic trace analysis.

The other approach more often adopted, consists in using several independent methods. Certification becomes possible and reliable when sufficient evidence is available that two or more independent methods give the same result. Each method should be applied in three or more laboratories of proven high quality. It is clear that the laboratories have to demonstrate beforehand that they work with methods which have been validated in detail, and particularly that proper calibration is performed to avoid systematic errors. This includes the calibration of balances, volumetric glassware and other tools of relevance, the use of calibrants of adequate purity and of known stoichiometry [17] and of proper solvents and reagents. Special precautions need to be taken when all laboratories use the same calibrant, e.g. because only one supplier exists. Chemical reaction yields should be known accurately and all precautions should be taken to avoid losses (e.g. from the formation of insoluble or volatile compounds, or incomplete extraction) and contamination.

If the results from entirely different methods, such as IDMS, INAA, ICP-MS, ICP-OES or atomic absorption spectrometry (AAS), and differential pulse anodic stripping voltammetry (DPASV) (between-method bias), as applied in different laboratories working independently (between-laboratory bias), are in agreement it can be concluded that the bias of each method is negligible and the mean value of the results is the best approximation to the true value. The remaining differences between laboratories are considered to be representative of the different sources of inaccuracy which still exist in the state of the art, and form a reliable basis for the determination of the uncertainty of the certified value.

11.6.5. Availability

Certified reference materials can really fulfil their task when they can be introduced into the quality assurance and quality control schemes set up by the laboratories. This can only be achieved when the analysts know that the CRMs will be available over long periods of time. The long term availability should oblige the producers to foresee batches of CRMs which are large enough to be available over several years and to replace exhausted CRMs by similar materials. However, replacement of CRMs is not easy as the freshly collected material may differ in composition from the previous CRM. An example of this situation has been encountered recently by the BCR (Bureau Communautaire de Référence) when it was necessary to replace a sewage sludge material certified for toxic elements (CRM 145). It was not possible to find a material with exactly the same trace element pattern. In particular, the Ni and the Cd contents in the prospective materials were always much lower than in the previous CRM 145, which was collected 15 years ago. This may reflect the actual contamination of sewage sludge by these elements. Therefore the new material may better reflect the situation encountered with natural samples analysed by the laboratories.

11.7. CRMS FOR ENVIRONMENTAL MONITORING

11.7.1. Suppliers

There are a number of suppliers of CRMs for environmental monitoring. Some of them specialize in a certain field of interest (e.g. the National Research Council of Canada - NRCC - for marine monitoring). Two main bodies, the National Institute of Standards and Technology (NIST, USA) and the BCR of the Commission of the European Communities, cover several fields and ensure long-term availability of CRMs, owing to the large batches of materials produced. The International Atomic Energy Agency (IAEA) in Vienna (Austria) mainly provides certified materials for nuclear measurements but also has available some CRMs for non-nuclear analysis. The ISO Council on Reference Materials (REMCO) of ISO has available a Directory for Reference Materials which may be consulted [33]. The International Union for Pure and Applied Chemistry (IUPAC) issues a catalogue of available CRMs [34]. Some additional compilations of existing CRMs in more specialized fields also exist [35]. The major source of information on reference

TABLE 11.1

EXAMPLES OF SOME RECENT CRMS FOR THE QUALITY CONTROL OF MEASUREMENTS IN ENVIRONMENTAL MONITORING (NON NUCLEAR FIELD AND ONLY CERTIFIED MATRIX MATERIALS)^a

Type	CRM No. ^b	Certified parameter	Supplier	Reference
Sediments				
Estuarine	CRM 277	Trace elements	BCR	37
Lake	CRM 280	Trace elements	BCR	37
River	CRM 320	Trace elements	BCR	37
Estuarine	SRM 1646	Trace elements	NIST	32
River	SRM 2704	Trace elements	NIST	32
River	SRM 1939	PCBs	NIST	32
Marine	HS 3-6	PAHs	NRCC	35
Marine	PACS-1	Tributyltin + trace elements	NRCC	35
Soils				
Loam	CRM 141	Trace elements	BCR	37
Sandy	CRM 142R	Trace elements	BCR	37
Amended	CRM 143R	Trace elements	BCR	37
Sewage sludges				
Domestic	CRM 144	Trace elements	BCR	37
Mixed origin	CRM 145R	Trace elements	BCR	37
Industrial	CRM 146	Trace elements	BCR	37
Mixed origin	CRM 392	PCBs	BCR	37
Mixed origin	CRM 088	PAHs	BCR	37
Ashes and dust				
Coal fly ash	CRM 038	Trace elements	BCR	37
Incineration	CRM 176	Trace elements	BCR	37
Fly ash extr.	CRM 429	PCDDs and PCDFs	BCR	37
Coal fly ash	SRM 1633a	Trace elements	NIST	32
Coal fly ash	SRM 2689-91	Major elements	NIST	32
Urban dust	SRM 1649	PAHs	NIST	32
Diesel partic.	SRM 1650	PAHs	NIST	32
Waters				
Fresh water	CRM 398	Trace and major	BCR	37
	CRM 399	compounds	BCR	37
Sea water	CRM 403	Trace elements	BCR	37
Water	SRM 1643b	Trace elements	NIST	32
Sea water	CASS-2	Trace elements	NRCC	35
	NASS-2	Trace elements	NRCC	35
	SLEW-1	Trace elements	NRCC	35
Rain water	CRM 408-9	Major compounds	BCR	37
Water	V-SMOW	O & H stable isotope ratios	IAEA	32

TABLE 11.1 (*continued*)

Type	CRM No. ^b	Certified parameter	Supplier	Reference
Waste				
Mineral oils	CRM 420-449	PCBs	BCR	37
Gases				
On Tenax	CRM 112	Benzene, toluene, <i>m</i> -xylene	BCR	37
In air	SRM 1670-72	CO ₂	NIST	32
In air	SRM 2607-10	CO ₂ – NO ₂	NIST	32
Animal tissues				
Mussel tissue	CRM 278	Trace elements	BCR	37
Cod muscle	CRM 422	Trace elements	BCR	37
Milk powder	CRM 063R	Trace elements	BCR	37
Human hair	CRM 397	Trace elements	BCR	37
Oyster tissue	SRM 1566a	Trace elements	NIST	32
Lobster	TORT-1	Trace elements	NRCC	35
Dogfish	DOLT-1	Trace elements	NRCC	35
	DORM-1	Trace elements	NRCC	35
Cod liver oil	CRM 349	PCBs	BCR	37
Mackerel oil	CRM 350	PCBs	BCR	37
Milk powder	CRM 450	PCBs	BCR	37
Cod liver oil	SRM 1588	PCBs+OCPs	NIST	32
Mussel tissue	SRM 1974	PAHs	NIST	32
Pork fat	CRM 430	OCPs	BCR	37
Milk powder	CRM 187-188	OCPs	BCR	37
Plant tissues				
Aquatic	CRM 060	Trace elements	BCR	37
Aquatic	CRM 061	Trace elements	BCR	37
Marine	CRM 279	Trace elements	BCR	37
Olive leaves	CRM 062	Trace elements	BCR	37
Hay powder	CRM 129	Trace elements	BCR	37
Rye grass	CRM 281	Trace elements	BCR	37
White clover	CRM 402	Trace elements	BCR	37
Plankton	CRM 414	Trace elements	BCR	37
Spruce needles	CRM 101	Nutrients and	BCR	37
Beech leaves	CRM 100	contaminants	BCR	37
Citrus leaves	SRM 1572	Trace elements	NIST	32
Pine needles	SRM 1575	Trace elements	NIST	32
Sargasso seaweed	No. 9	Trace elements	NIES	35

^aNIES, National Institute for Environmental Studies of Japan; PAH, polycyclic aromatic hydrocarbons; OCP, organochlorine pesticides.

^bCertified following the ISO Guide 35-1985 [31].

materials is the COMAR Data Bank which is based at the Laboratoire National d'Essais in France.

11.7.2. Types of CRMs

CRMs are products of very high added value. Their production is very costly (some hundred thousands of US dollars) and therefore they should be reserved for selected tests as final verification of the analytical procedure. The CRMs for environmental monitoring mainly concern matrix materials certified for chemical or biochemical content, but sometimes physical parameters (e.g. the conductance of rainwater, NIST SRM 2694). Reference materials certified for microbiological parameters are being prepared by the BCR [36], mainly for the quality control of microbiological determinations in water and food.

Table 11.1 gives a list of some CRMs already available from various suppliers in 1992. This list covers only matrix materials in the non-nuclear field and is not exhaustive. For more updated information on the CRMs the user should contact the producers [32,35,37]. Some animal tissues or products or plants are listed here as they are often used to evaluate the global contamination of the environment (target animals or plants) or because they are representative of the contamination in the food chain.

Several industrial products, e.g. coals, may serve for the economic evaluation of materials as well as for the control of potential contamination by some toxic compounds or elements. Therefore, they have some interest for environmental monitoring but have not been listed in the table.

11.8. CONCLUSION

Certified reference materials are keys to the achievement of reliable, accurate results which are the basis of the proper monitoring of the environment and consequently for proper decisions to be taken to maintain or improve the prevailing situation. The use of CRMs in laboratories ensures the possibility of providing accurate results which are also traceable to recognized international references (standards) and therefore can be compared with the results from any other laboratory having the same traceability.

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Chapter 12

Standard reference materials for the determination of trace organic constituents in environmental samples

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12.1. INTRODUCTION

Two of the most widely measured groups of environmental contaminants are polycyclic aromatic compounds (PACs), particularly polycyclic aromatic hydrocarbons (PAHs) and organochlorine compounds, primarily polychlorinated biphenyls (PCBs) and chlorinated pesticides. PACs are widespread environmental pollutants resulting from emissions from a variety of sources including: industrial combustion and discharge of fossil fuels; residential heating (both fossil fuels and wood burning); automobile exhaust. Because of their mutagenic and carcinogenic properties, PACs have been measured in a variety of environmental matrices including air, water, soil (sediment) and tissue samples. PACs are

usually present in environmental samples as extremely complex mixtures. These mixtures contain many isomeric structures and alkylated isomers which vary greatly in relative concentrations of the individual components and in carcinogenic and/or mutagenic properties. PCBs and chlorinated pesticides are also widespread pollutants, particularly in the marine environment where organisms tend to concentrate these highly lipophilic compounds in the fatty tissues. PCB mixtures in environmental samples generally consist of about 100 congeners (of the 209 possible congeners) which vary in relative concentration and toxicity.

Laboratories worldwide are involved in the analysis of environmental samples for the determination of these organic contaminants. The accurate determination of organic contaminants is a difficult analytical challenge since the analytes of interest are generally present at trace levels ($\mu\text{g/g}$ or less) in complex natural matrices such as sediment or biological tissue, and they are present as complex mixtures of numerous isomers or congeners, as mentioned above, which requires highly efficient and/or selective separations. Obtaining reliable data from the analysis of environmental samples requires the use of analytical methods that have been validated as to their accuracy, and these analytical methods must be continuously monitored to verify that they remain in control. One method for validating analytical procedures is by analyzing reference materials that have been well characterized with respect to the analytes of interest. Environmental matrix reference materials that are certified for trace element content have been available from several organizations for a number of years [1–5]. However, reference materials for the determination of organic contaminants such as PAHs, PCBs and chlorinated pesticides have only become widely available in recent years. Since 1980, the National Institute of Standards and Technology (NIST), formerly the National Bureau of Standards, has issued a number of Standard Reference Materials (SRMs) for use in the determination of organic constituents in environmental samples. These SRMs have focused primarily on organic contaminants such as PAHs, PCBs and chlorinated pesticides. A review of the SRM activities for PAH measurements at NIST in 1988 has been published [6]. A large number of gas standards for environmental measurements are also available from NIST but are not discussed in this paper; a review of the gas standards for environmental analysis has been published [7].

The first matrix SRMs developed at NIST for organic contaminant measurements focused on PAH measurements in fossil fuels, i.e. shale oil [8,9] and petroleum crude oil [10,11], followed by PAHs in air and diesel particulate material [12]. The most recent natural matrix SRMs for PAH measurements have focused on the marine environment with sediment [13] and mussel tissue samples [14]. The first SRMs for PCB measurements were natural matrices (oil and human serum) that had been fortified with PCB mixtures and quantified as Aroclor mixtures; however, recent efforts in PCB measurements have been directed toward individual PCB congener measurements in natural marine samples such as fish oil, sediment, mussel tissue and whale blubber. The marine sediment and mussel tissue materials were also the first materials to be analyzed for both PAHs and organochlorine compounds. A review of the NIST SRMs for organic analyses related to the marine environment has been published recently [15]. In this chapter, the NIST SRMs available for use in the determination of organic contaminants in environmental samples are described with particular emphasis on recent materials related to contaminants in the marine environment.

12.2. NIST SRMS FOR ORGANIC CONTAMINANTS

Reference materials are used primarily for the following purposes: (1) to calibrate the measurement system; (2) to validate the reliability and precision of a new analytical method; (3) to provide quality control of routine analyses by analyzing the SRM at appropriate, regular time intervals. The NIST SRMs for organic environmental analyses are summarized in Tables 12.1 and 12.2. The SRMs in Tables 12.1 and 12.2 represent two different levels of analytical difficulty: (1) simple calibration solutions containing a number of analytes and (2) natural matrix materials. The calibration solutions (see Table 12.1) are useful for validating the chromatographic separation step (e.g. retention times and analyte detector response); whereas the natural matrix materials (see Table 12.2), which are similar to the actual environmental samples analyzed, can be used to validate the complete analytical procedure including extraction, isolation/clean-up procedures, and the final chromatographic separation and quantification.

For each of the SRMs listed in Tables 12.1 and 12.2, information is provided on the number of analytes for which "certified" and "non-certified" concentration values are provided in the Certificate of Analysis that accompanies each SRM sample. The certified values for each of these SRMs are based on the agreement of results obtained from two or more independent and reliable analytical procedures, whereas the non-certified values are generally results from only one analytical technique. The analytical techniques used for the determination of the certified and non-certified values for these SRMs are summarized in Table 12.3 for PAC analytes and in Table 12.4 for the organochlorine compounds. For the calibration solution SRMs in Tables 12.3 and 12.4, the certified values are generally based on accurately weighed quantities of the analytes and solvent used to prepare the solution and on measurements obtained using liquid chromatography (LC), gas chromatography (GC) with flame ionization detection (FID) and electron capture detection (ECD) and/or gas chromatography-mass spectrometry (GC-MS). The natural matrix materials present a more difficult analytical task that usually requires extraction of the matrix to remove the organic compounds, clean-up procedures to isolate the compounds of interest, and finally, analysis using GC or LC with selective detection to measure individual compounds in the complex natural mixtures. The following analytical techniques were used for the PAH measurements in natural matrix SRMs: reversed-phase LC with fluorescence detection (LC-FL); GC-FID; GC-MS. For PCB and pesticide measurements (Table 12.4), GC-ECD has been the primary technique with GC-MS used recently as the second technique. To satisfy the requirement of independent analytical procedures for the certification of natural matrix SRMs, the extraction and clean-up/isolation steps that precede the final chromatographic measurements should also be based on different separation characteristics to minimize the possibility of similar biases in both measurements. The different analytical techniques used and the results are discussed below.

12.2.1. Calibration solutions

The calibration solution SRMs, which represent the samples of least analytical difficulty, are useful for several purposes including: (1) calibration of chromatographic instrumentation for retention times and detector response factors for quantitation; (2) spik-

TABLE 12.1

NIST CALIBRATION SOLUTION SRMs FOR THE DETERMINATION OF ORGANIC CONTAMINANTS

SRM no.	Title	Date issued	Certified constituents	Non-certified constituents
1491	Aromatic Hydrocarbons in Hexane/ Toluene	1989	PAHs (23)	PAHs (1)
1492	Chlorinated Pesticides in Hexane	1992	Pesticides (15)	
1493	Chlorinated Biphenyl Congeners in 2,2,4- Trimethylpentane	1993	PCBs (18)	PCBs (2)
1494	Aliphatic Hydrocarbons in 2,2,4-Tri- methylpentane	1993	Hydrocarbons (20)	
1583	Chlorinated Pesticides in 2,2,4-Tri- methylpentane	1985	Pesticides (5)	Pesticides (1)
1584	Priority Pollutant Phenols in Methanol	1984	Phenols (10)	Phenols (1)
1585	Chlorinated Biphenyls in 2,2,4-Tri- methylpentane	1986	PCBs (8)	
1586	Isotopically Labeled and Unlabeled Priority Pollutants in Methanol	1984	Priority pollu- tants (10)	
1587	Nitrated PAHs in Methanol	1985	Nitro-PAHs (6)	Nitro-PAHs (1)
1596	Dinitropyrene Isomers and 1-Nitropyrene in Methylene Chloride	1987	Nitro-PAHs (4)	
1614	Dioxin (2,3,7,8-TCDD) in Iso-octane	1985	Dioxins (2)	Dioxins (2)
1639	Halocarbons (in Methanol) for Water Analysis	1983	Halocarbons (7)	
1644	Generator Columns for PAHs	1981	PAHs (3)	
1647c	Priority Pollutant PAHs (in Acetonitrile)	1992	PAHs (16)	
2260	Aromatic Hydrocarbons in Toluene (nominal concentration 60 $\mu\text{g/ml}$)	1991	PAHs (23)	PAHs (1)
2261	Chlorinated Pesticides in Hexane (nominal concentration 2 $\mu\text{g/ml}$)	1992	Pesticides (15)	
2262	Chlorinated Biphenyl Congeners in 2,2,4- Trimethylpentane (nominal concentra- tion 2 $\mu\text{g/ml}$)	1993	PCBs (25)	PCBs (3)

ing or fortifying samples; (3) analyte recovery studies; (4) determining method response factors. NIST has issued 17 different calibration solution SRMs since 1981 as shown in Table 12.1. The most recent emphasis has focused on four groups of organic contaminants routinely measured in marine monitoring programs (i.e. PAHs, PCBs, chlorinated pesticides and aliphatic hydrocarbons). The compounds and concentration ranges of the ten calibration solution SRMs for these four groups of analytes are summarized in Table 12.5. The compounds used to prepare these calibration solutions are obtained in the highest purity available (preferably certified reference materials where available); if the purity

TABLE 12.2

NIST NATURAL MATRIX SRMs FOR THE DETERMINATION OF ORGANIC CONTAMINANTS IN ENVIRONMENTAL SAMPLES^a

SRM no.	Title	Date issued	Certified constituents	Non-certified constituents
1580	Organics in Shale Oil	1980	PAHs (5); phenols (3); PANH (1)	Phenols (6); PANH (1)
1581	Polychlorinated Biphenyls in Oils	1982	Aroclor 1242/1260	
1582	Petroleum Crude Oil	1984	PAHs (5); PASH (1)	PAHs (5); phenols (2); PANH (1)
1588	Organics in Cod Liver Oil	1988	PCBs (5); pesticides (10)	PCDDs/PCDFs (7)
1589	Polychlorinated Biphenyls (as Aroclor 1260) in Human Serum	1985	Aroclor 1260	Dioxins (2)
1597	Complex Mixture of PAHs from Coal Tar	1987	PAHs (12)	PAHs/PACs (18)
1648	Urban Particulate Matter	1978	Trace elements (9)	Trace elements (25); PAH (13)
1649	Urban Dust/Organics	1982	PAHs (5)	PAHs (9)
1650	Diesel Particulate Material	1985	PAHs (5); nitro-PAHs (1)	PAHs (6); nitro-PAHs (3); PAQ (1)
1939	Polychlorinated Biphenyls (Congeners) in River Sediment	1990	PCBs (3)	PCBs (14); pesticides (5); PAHs (5)
1941	Organics in Marine Sediment	1989	PAHs (11)	PAHs (24); PCBs (15); pesticides (7); trace elements (32)
1945	Organics in Whale Blubber (in preparation)	1993	PCBs; pesticides	
1974	Organics in Mussel Tissue (<i>Mytilus edulis</i>)	1990	PAHs (9)	PAHs (19); PCBs (13); pesticides (9); trace elements (34)
1975	Diesel Particulate Extract (in preparation)	1993	PAHs; nitro-PAHs	

^aPANH, polycyclic aromatic nitrogen heterocycles; PAQ, polycyclic aromatic quinones; PASH, polycyclic aromatic sulfur heterocycles; PCDD, polychlorinated dibenzodioxins; PCDF, polychlorinated dibenzofurans.

of the material is unknown, analyses are performed using GC, LC, and/or differential scanning calorimetry to establish the purity.

One of the first and by far the most popular of the organic environmental SRMs is SRM 1647, an acetonitrile solution of the 16 PAHs on the US Environmental Protection Agency's (EPA) priority pollutant list. This SRM was prepared at the request of EPA in support of EPA Method 610, which specifies the use of reversed-phase LC with fluorescence detection for the determination of PAHs. SRM 1647 has found widespread use as a calibration solution to determine retention times and detector response factors in LC. Because of the popularity of SRM 1647, it has been reissued three times since first issued in 1981 and is now available as SRM 1647c. The chromatogram for the reversed-phase

TABLE 12.3

ANALYTICAL METHODS USED FOR THE CERTIFICATION OF SRMs FOR THE DETERMINATION OF PAC^a

Calibration solution SRMs

1491	Aromatic Hydrocarbons in Hexane/Toluene	Gravimetry	GC-FID
1644	Generator Columns for PAHs	LC-UV [16,17]	Fluorescence [18]
1647c	Priority Pollutant PAHs in Acetonitrile	Gravimetry	LC-UV
1587	Nitrated PAHs in Methanol	LC-UV	GC-FID
1596	Dinitropyrene Isomers and 1-Nitropyrene in Methylene Chloride	LC-UV	GC-MS

Natural matrix SRMs

1580	Organics in Shale Oil	LC-FL [8,9] LC-UV (phenols) [8]	GC-MS [8,9]
1582	Petroleum Crude Oil	LC-FL [11]	GC-MS [10] LC-EC (phenols) [21]
1597	Complex Mixture of PAHs from Coal Tar	LC-FL [23]	GC-FID [23]
1648	Urban Particulate Matter	LC-FL [12,24]	GC-FID [12,24]
1649	Urban Dust/Organics	LC-FL [12,24]	GC-FID [12,24]
1650	Diesel Particulate Material	LC-FL LC-EC (nitro-PAH) [26]	GC-MS [25] LC-FL (nitro-PAH) [26]
1941	Organics in Marine Sediment	LC-FL [13]	GC-FID [13] GC-MS [13]
1974	Organics in Mussel Tissue (frozen)	LC-FL [14]	GC-MS [14]
1975	Diesel Particulate Extract (in preparation)	LC-FL	GC-MS

^aAnalytical methods are described briefly in Certificate of Analysis for each SRM; references listed describe methods and results in more detail. GC-FID, gas chromatography-flame ionization detection; GC-FPD, gas chromatography-flame photometric detection; LC-UV, liquid chromatography-ultraviolet detection; GC-MS, gas chromatography-mass spectrometry; LC-FL, liquid chromatography-fluorescence detection; LC-EC, liquid chromatography-electrochemical detection.

TABLE 12.4

ANALYTICAL METHODS USED FOR THE CERTIFICATION OF SRMs FOR THE DETERMINATION OF PCBs AND CHLORINATED PESTICIDES^a

Calibration solution SRMs			
1492	Chlorinated Pesticides in Hexane	Gravimetry	GC-ECD
1493	Chlorinated Biphenyl Congeners in 2,2,4-Trimethylpentane	Gravimetry	GC-ECD
1583	Chlorinated Pesticides in 2,2,4-Trimethylpentane	Gravimetry	GC-ECD
1584	Priority Pollutant Phenols in Methanol	Gravimetry	GC-FID, LC-UV
1585	Chlorinated Biphenyls in 2,2,4-Trimethylpentane	Gravimetry	GC-ECD
1586	Isotopically Labeled and Unlabeled Priority Pollutants	Gravimetry	GC-FID
1614	Dioxin (2,3,7,8-TCDD) in Iso-octane	Gravimetry	GC-ECD
1639	Halocarbons (in Methanol) for Water Analysis	Gravimetry	GC-ECD, GC-HECD
2261	Chlorinated Pesticides in Hexane	Gravimetry	GC-ECD
2262	Chlorinated Biphenyl Congeners in 2,2,4-Trimethylpentane	Gravimetry	GC-ECD
Natural matrix SRMs			
1581	Polychlorinated Biphenyls in Oils	Gravimetry	GC-ECD
1588	Organics in Cod Liver Oil	GC-ECD	GC-MS
1589	Polychlorinated Biphenyls (as Aroclor 1260) in Human Serum	Gravimetry	GC-ECD
1939	Polychlorinated Biphenyls (Congeners) in River Sediment	GC-ECD [29]	GC-MS [29]
1941	Organics in Marine Sediment	GC-ECD [13]	
1974	Organics in Mussel Tissue (<i>Mytilus edulis</i>)	GC-ECD [14]	GC-MS [14]
1945	Organics in Whale Blubber (in preparation)	GC-ECD	GC-MS

^aAnalytical methods are described briefly in Certificate of Analysis for each SRM; references listed describe methods and results in more detail. GC-ECD, gas chromatography-electron capture detection; GC-FID, gas chromatography-flame ionization detection; GC-HECD, gas chromatography-Hall electrolytic conductivity detection; LC-UV, liquid chromatography-ultraviolet detection; GC-MS, gas chromatography-mass spectrometry.

LC analysis of SRM 1647c is shown in Fig. 12.1. Since SRM 1647c is used primarily for calibration of reversed-phase LC instrumentation and spiking of aqueous-based matrices (i.e. because of the acetonitrile solvent), two similar solutions (SRMs 1491 and 2260) have been prepared in hexane and/or toluene to provide a solvent more compatible with GC and normal-phase LC analyses and for spiking into non-aqueous matrices. In addition to the 16 PAHs included in SRM 1647, SRMs 1491 and 2260 contain eight additional PAH analytes (see Table 12.5), which were included in the SRMs specifically to meet the needs of a national marine pollution monitoring program in the United States (i.e. the National Status and Trends Program) sponsored by the US National Oceanic and Atmospheric Administration (NOAA). SRMs 1491 and 2260 contain the same 24 analytes but

TABLE 12.5

ANALYTES IN NIST CALIBRATION SOLUTION SRMs FOR ALIPHATIC HYDROCARBONS, PAHs, PCBs AND CHLORINATED PESTICIDES

Polycyclic aromatic hydrocarbons in SRMs 1491 and 2260 (~4–8 and 50–70 µg/ml)	Aliphatic hydrocarbons in SRM 1494 (~6–130 µg/ml)	Polychlorinated biphenyl congeners in SRMs 1493 and 2262 (~0.2 and 2 µg/ml)		Chlorinated pesticides in SRMs 1492 and 2261 (~0.2 and 2 µg/ml)
Naphthalene ^a	<i>n</i> -Decane (<i>n</i> -C ₁₀)	PCB 1	2-Chlorobiphenyl ^c	Hexachlorobenzene
2-Methylnaphthalene	<i>n</i> -Undecane (<i>n</i> -C ₁₁)	PCB 8	2,4'-Dichlorobiphenyl	γ-HCH ^d
1-Methylnaphthalene	<i>n</i> -Dodecane (<i>n</i> -C ₁₂)	PCB 18	2,2',5-Trichlorobiphenyl	Heptachlor
Biphenyl	<i>n</i> -Tridecane (<i>n</i> -C ₁₃)	PCB 28	2,4,4'-Trichlorobiphenyl ^b	Aldrin ^d
2,6-Dimethylnaphthalene	<i>n</i> -Tetradecane (<i>n</i> -C ₁₄)	PCB 29	2,4,5-Trichlorobiphenyl ^c	Heptachlor epoxide ^d
Acenaphthylene ^a	<i>n</i> -Pentadecane (<i>n</i> -C ₁₅)	PCB 44	2,2',3,5'-Tetrachlorobiphenyl	<i>cis</i> -Chlordane
Acenaphthene ^a	<i>n</i> -Hexadecane (<i>n</i> -C ₁₆)	PCB 50	2,2',4,6-Tetrachlorobiphenyl ^c	<i>trans</i> -Nonachlor
1,6,7-Trimethylnaphthalene	<i>n</i> -Heptadecane (<i>n</i> -C ₁₇)	PCB 52	2,2',5,5'-Tetrachlorobiphenyl ^b	Dieldrin
Fluorene ^a	<i>n</i> -Octadecane (<i>n</i> -C ₁₈)	PCB 66	2,3',4,4'-Tetrachlorobiphenyl	Mirex
Phenanthrene ^a	<i>n</i> -Nonadecane (<i>n</i> -C ₁₉)	PCB 77	3,3',4,4'-Tetrachlorobiphenyl ^b	2,4'-DDE
Anthracene ^a	<i>n</i> -Eicosane (<i>n</i> -C ₂₀)	PCB 87	2,2',3,4,5'-Pentachlorobiphenyl ^c	4,4'-DDE ^d
1-Methylphenanthrene	<i>n</i> -Docosane (<i>n</i> -C ₂₂)	PCB 101	2,2',4,5,5'-Pentachlorobiphenyl ^b	2,4'-DDD

Fluoranthene ^a	<i>n</i> -Tetracosane (<i>n</i> -C ₂₄)	PCB 104	2,2',4,6,6'-Pentachlorobiphenyl ^c	4,4'-DDD
Pyrene ^a	<i>n</i> -Hexacosane (<i>n</i> -C ₂₆)	PCB 105	2,3,3',4,4'-Pentachlorobiphenyl	2,4'-DDT
Benz[<i>a</i>]anthracene ^a	<i>n</i> -Octacosane (<i>n</i> -C ₂₈)	PCB 118	2,3',4,4',5-Pentachlorobiphenyl	4,4'-DDT ^d
Chrysene ^a	<i>n</i> -Triacotane (<i>n</i> -C ₃₀)	PCB 126	3,3',4,4',5-Pentachlorobiphenyl	
Benzo[<i>b</i>]fluoranthene ^a	<i>n</i> -Dotriacontane (<i>n</i> -C ₃₂)	PCB 128	2,2',3,3',4,4'-Hexachlorobiphenyl	
Benzo[<i>k</i>]fluoranthene ^a	<i>n</i> -Tetraatriacontane (<i>n</i> -C ₃₄)	PCB 138	2,2',3,4,4',5'-Hexachlorobiphenyl ^b	
Benzo[<i>e</i>]pyrene ^a	Pristane	PCB 153	2,2',4,4',5,5'-Hexachlorobiphenyl ^b	
Benzo[<i>a</i>]pyrene ^a	Phytane	PCB 154	2,2',4,4',5,6'-Hexachlorobiphenyl ^c	
Perylene ^a		PCB 170	2,2',3,3',4,4',5-Heptachlorobiphenyl	
Indeno[1,2,3- <i>cd</i>]pyrene ^a		PCB 180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	
Dibenz[<i>a,h</i>]anthracene ^a		PCB 187	2,2',3,4',5,5',6-Heptachlorobiphenyl	
Benzo[<i>ghi</i>]perylene ^a		PCB 188	2,2',3,4',5,6,6'-Heptachlorobiphenyl ^c	
		PCB 195	2,2',3,3',4,4',5,6-Octachlorobiphenyl	
		PCB 201	2,2',3,3',4,5',6,6'-Octachlorobiphenyl ^c	
		PCB 206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	
		PCB 209	Decachlorobiphenyl	

^aAnalytes in SRM 1647c (~1–20 µg/ml).

^bAnalytes in SRM 1585 (~1–30 µg/ml). PCB 3 (4-chlorobiphenyl) and PCB 15 (4,4'-dichlorobiphenyl) are also in SRM 1585.

^cAnalytes not in SRM 1493.

^dAnalytes in SRM 1583 (~0.5–1.3 µg/ml). γ-HCH is also in SRM 1583.

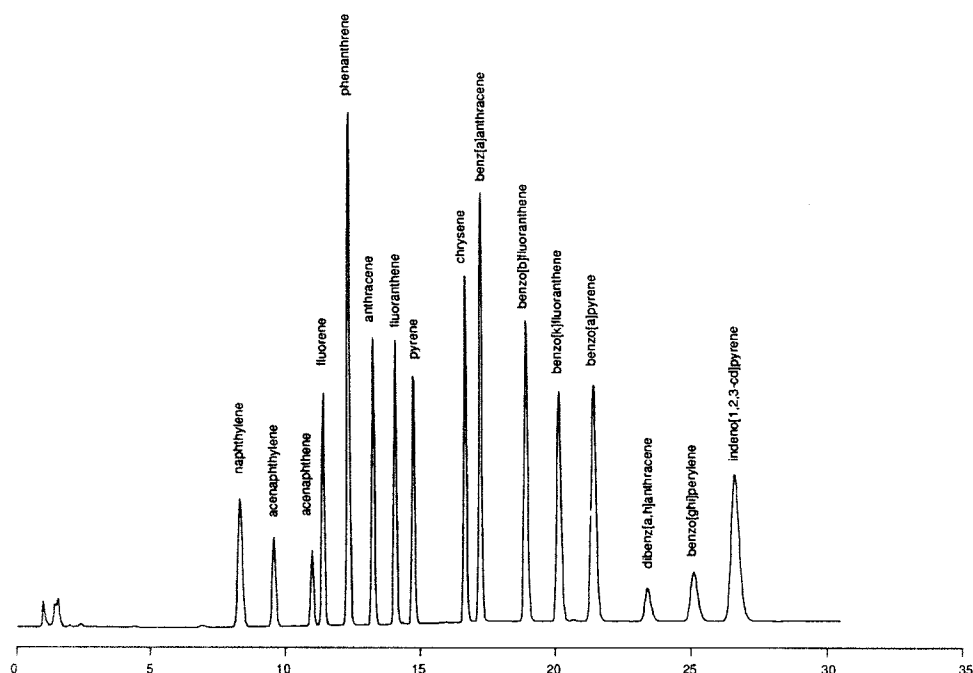


Fig. 12.1. Reversed-phase liquid chromatographic analysis of SRM 1647c. Column: C-18 column (Hypersil PAH). Mobile phase conditions: 50% acetonitrile in water for 4 min, then linear gradient to 100% acetonitrile in 10 min and hold.

at concentrations that differ by approximately a factor of ten. SRMs 1491 and 2260 can be diluted to produce multipoint calibration curves. SRM 1494 (Aliphatic Hydrocarbons in 2,2,4-Trimethylpentane) was developed for use in the measurement of aliphatic hydrocarbons, particularly for assessment of petroleum contamination in the marine environment. SRM 1494 contains *n*-alkanes from C₁₀ to C₂₀, even number *n*-alkanes from C₂₂ to C₃₄, pristane and phytane. Pristane and phytane are often measured for use as marker compounds to determine whether the input of hydrocarbons in the sample is of petrogenic or natural origin.

In 1985 and 1986, calibration solution SRMs were issued containing six chlorinated pesticides (SRM 1583) and eight PCB congeners (SRM 1585). Recently, these SRMs have been superseded by calibration solution SRMs that contain more analytes, i.e. SRMs 1492 and 2261 which each contain 15 chlorinated pesticides and SRMs 1493 and 2262 which contain 20 and 28 PCB congeners, respectively. The number of analytes in these recent calibration solution SRMs was expanded to meet the needs of the NOAA marine monitoring program; eight additional PCB congeners were added to SRM 2262 at the request of the EPA. The PCB congeners and chlorinated pesticide SRMs are issued at two concentration levels (approximately 0.2 and 2 µg/ml) for use in preparing multipoint calibration curves.

In addition to the calibration solution SRMs described in Table 12.5 related to aliphatic hydrocarbons, PAHs, PCBs and chlorinated pesticides, seven additional solution SRMs have been issued for different classes of organic contaminants. SRM 1644 (Generator Columns for PAHs) is a unique SRM for preparing calibration solutions of PAHs in water. SRM 1644 consists of three individual coiled stainless steel tubes containing sand that has been coated with approximately 0.5% by weight of anthracene, benz[*a*]anthracene or benzo[*a*]pyrene. By passing high purity water slowly through the column, a saturated aqueous solution is obtained. Since the aqueous solubility of a compound is a well-defined thermodynamic quantity, this saturated solution has a known concentration at a specific temperature.

The determination of nitrated PAHs in environmental samples has become important in recent years because of the high direct-acting mutagenicity of some of these compounds. Two calibration SRMs containing nitrated PAHs have been issued by NIST: SRM 1587 (Nitrated Polycyclic Aromatic Hydrocarbons in Methanol); SRM 1596 (Dinitropyrene Isomers and 1-Nitropyrene in Methylene Chloride). SRM 1587 contains seven nitro-PAHs (2-nitrofluorene, 9-nitroanthracene, 3-nitrofluoranthene, 1-nitropyrene, 7-nitrobenz[*a*]anthracene, 6-nitrochrysene and 6-nitrobenzo[*a*]pyrene) at concentrations of approximately 4–7 µg/ml. SRM 1596 contains a mixture of three dinitropyrene isomers (1,3-, 1,6- and 1,8-dinitropyrene) and 1-nitropyrene at concentrations of approximately 3–10 µg/ml.

SRM 1584 (Priority Pollutant Phenols in Methanol) contains 10 of the 11 phenols on the EPA's list of priority pollutants at concentrations ranging from 15 to 65 µg/ml. SRM 1586 (Isotopically Labeled and Unlabeled Priority Pollutants in Methanol) was developed for use by laboratories using EPA Analytical Methods 1624 and 1625 (as well as 624–625 and 524–525). These methods specifically require the use of GC-MS and the use of isotopically labeled internal standards. SRM 1586 is composed of two separate solutions each containing the following compounds: carbon tetrachloride; benzene; chlorobenzene; phenol; nitrobenzene; 2-nitrophenol; 2,4-dichlorophenol; naphthalene; bis[2-ethylhexyl]-phthalate; benzo[*a*]pyrene. One solution contains the ten compounds with carbon-13 (carbon tetrachloride) or deuterium (all other compounds) labeled compounds whereas the other solution contains unlabeled compounds. The concentrations of the analytes range from approximately 40 to 130 µg/g. Another SRM containing labeled and unlabeled pollutants is SRM 1614 (Dioxin (2,3,7,8-TCDD) in Iso-octane). This SRM consists of two solutions, one containing ¹³C-labeled 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the second containing unlabeled 2,3,7,8-TCDD both at concentrations of approximately 65 ng/ml. Non-certified values are also reported for a labeled and unlabeled trichlorodibenzo-*p*-dioxin congener at 1–3 ng/ml levels. SRM 1639 (Halocarbons (in Methanol) for Water Analysis) is a methanol solution containing seven halocarbons: chloroform, chlorodibromomethane, bromodichloromethane, bromoform, carbon tetrachloride, trichloroethylene and tetrachloroethylene at concentrations ranging from 40 to 6200 ng/µl.

12.2.2. Natural matrix SRMs

Validation of the complete analytical procedure (including solvent extraction, clean-up of the extracts, isolation of the analytes of interest and chromatographic analysis) requires

TABLE 12.6

CONCENTRATIONS ($\mu\text{g/g}$) OF THE PAHs IN NATURAL MATRIX SRMs (FOSSIL FUELS AND PARTICULATE MATERIALS)^a

Compounds	Shale oil SRM 1580	Petroleum crude oil SRM 1582	Air particulate matter SRM 1648	Urban dust organics SRM 1649	Diesel particulate matter SRM 1650
Phenanthrene	232 (12) ^b 255 (8) ^c	101 \pm 5	3.8 (0.2) ⁱ 4.6 (0.3) ^j	4.5 (0.3) ^j	79 (1) ^k 63 (2) ^m
Anthracene	88.4 (2.6) ^b 85.9 (4.8) ^c		0.36 (0.1) ^j	0.58 (0.2) ^l 0.41 (0.44) ^j	0.88 (0.05) ^q
Fluoranthene	54 \pm 10	2.5 \pm 0.3	9.1 (0.8) ^k 9.2 (0.5) ⁱ 8.7 (0.4) ^j	7.1 \pm 0.5	51 \pm 4
Pyrene	104 \pm 18	8.0 (0.4) ^g 7.2 (0.2) ^g 6.8 (0.2) ^j	7.4 (0.6) ^k 7.3 (0.4) ⁱ	7.2 (0.2) 6.3 (0.4)	48 \pm 4
Benz[<i>a</i>]anthracene	35.2 (2.5) ^b 36.1 (3.2) ^c	3.0 \pm 0.3	3.2 (0.2) ^k 3.2 (0.2) ^j 3.2 (0.1) ^j	2.6 \pm 0.3	6.5 \pm 1.1
Chrysene	43.9 (3.4) ^{b,p} 41.0 (4.5) ^c		6.6 (0.2) ^j	3.5 (0.2)	22 (1) ^m
Benzo[<i>b</i>]fluoranthene	12 (2) ^d		8.0 (0.5) ^q	6.2 (0.3)	

Benzo[k]fluoranthene	5 (1) ^d		3.4 (0.5) ^j	2.0 (0.1)	2.0 (0.06) ^q
Benzo[a]pyrene	21 ± 6	1.1 ± 0.3	3.1 (0.2) ^k	2.9 ± 0.5	1.2 ± 0.3
			3.1 (0.2) ^j		
			3.4 (0.4) ^j		
Benzo[e]pyrene	18 ± 8	3.5 (0.5) ^b	5.2 (0.3) ^k	3.3 (0.2) ⁱ	9.6 (0.3) ^k
	18.8 (0.8) ^b		5.2 (0.3) ⁱ		7.3 (1.2) ^l
Perylene	3.4 ± 2.2^c	31 ± 3	0.76 (0.07) ⁱ	0.84 (0.09) ⁱ	0.13 (0.02) ^m
	2.5 (0.2) ^f		0.69 (0.02) ^j	0.65 (0.2) ^j	
Benzo[ghi]perylene	7.1 (0.8) ^b	1.7 (0.04) ^b	5.3 (0.4) ^k	4.5 ± 1.1	2.4 ± 0.6
			4.7 (0.1) ⁱ		
Indeno[1,2,3- <i>cd</i>]pyrene	4.6 (0.6) ^b	0.17 (0.04) ^b	4.8 (0.2) ^k	3.3 ± 0.5	1.8 (0.1) ^k
			4.4 (0.15) ⁱ		2.1 (0.1) ^k
			4.7 (0.2) ^j		3.2 (0.3) ^m

^aCertified concentrations in bold (see Certificate of Analysis). The certified values were obtained from the combined results of at least two analytical results and the associated uncertainties are generally expressed as ± two standard deviations of the mean values of the two techniques or the 95% confidence intervals of each of the techniques. Non-certified values are indicated in parentheses (see Certificate of Analysis or literature reference indicated); uncertainties of the values in parentheses are ± one standard deviation of the mean value of replicate analyses and recognize only the within method variability. ^bGC-MS electron impact [30]. ^cDetermined by LC-FL [31] using methodology described in [11]. ^dLC quenchofluorometric detection [32]. ^eLC value used in certification (3.9 ± 0.6 µg/g) contained a contribution from benzo[k]fluoranthene which co-eluted with perylene on the column used. GC-MS value was 2.8 ± 0.6 µg/g. ^fLC-FL [11]. ^gGC-MS methane positive chemical ionization. ^hGC-MS methane negative ion chemical ionization [10]. ⁱDetermined by GC [12]. ^jLC-FL. ^kGC-MS negative ion chemical ionization [25]. ^lGC [33]. ^mLC-FL. ⁿLC-FL [23]. ^oGC [24]. ^pGC-MS value contains contribution from triphenylene. ^qLC-FL, data from [48].

the use of SRMs with matrices similar to those typically encountered in the analysis of environmental samples. Thus, the natural matrix SRMs are the most suitable materials for this purpose. Fourteen natural matrix SRMs are available from NIST with certified and non-certified concentrations of PACs, aliphatic hydrocarbons, phenols, PCBs and chlorinated pesticides (see Table 12.2).

12.2.2.1. Natural matrix SRMs for PAC determination

Since 1980, seven natural matrix SRMs have been issued with certified concentrations of PAHs and other PACs, i.e. SRMs 1580, 1582, 1597, 1649, 1650, 1941 and 1974). SRM 1648 (Urban Particulate Matter) was issued in 1978 and certified for inorganic constituents; however, data have been reported in the literature for PAH concentrations [24]. These SRMs represent several matrix types, relative PAH concentrations and sources of the PAH (i.e. petrogenic or pyrolytic). SRMs 1580 and 1582 are representative of oil matrices with petrogenic PAHs (i.e. formed from low-temperature processes) which have high levels of alkyl-substituted PAHs relative to the unsubstituted PAHs. These two materials have been described in more detail elsewhere [8–11,19,20]. SRMs 1648 and 1649 are two air particulate samples that were collected in the mid-1970s in St. Louis, MO and Washington, DC, respectively, and that have similar concentrations for the major PAH. An extensive characterization and comparison of the PAH content of these two air particulate SRMs have been reported in the literature [24]. The diesel particulate sample (SRM 1650) is representative of heavy duty diesel emissions. The concentrations of 13 common PAHs in the two fossil fuel and three particulate SRMs are summarized and compared in Table 12.6. In addition to the certified and non-certified concentrations of PAHs, Table 12.6 also contains results from additional analyses that have been performed at NIST on these materials since they were issued as SRMs, but are not reported on the Certificate of Analysis. (References to these additional measurements are provided in the footnotes in Table 12.6.)

SRM 1597 (Complex Mixture of PAHs from Coal Tar) is a natural pyrolytic PAH mixture (see discussion below) which is intermediate in complexity when compared to the calibration SRMs and the other natural matrix materials. The concentrations of 30 PAHs in SRM 1597 are provided in Table 12.7. The PAH mixtures from the two air particulate materials and the coal tar are representative of PAHs from pyrolytic sources (i.e. high temperature formation) which have low levels of alkylated PAHs relative to the unsubstituted PAHs. The PAHs in the diesel particulate SRM are a mixture of both pyrolytic and petrogenic (from residual fuel on the particles) PAHs as indicated by the relative high concentrations of the phenanthrene, pyrene and fluoranthene compared to the PAHs of higher molecular weight (see Table 12.6) and the relative high levels of methyl and dimethyl-substituted phenanthrenes [33].

The two most recent SRM matrices for PAH measurements are sediment and mussel tissue (see Table 12.8). SRM 1941 is a marine sediment that was collected in Baltimore Harbor (MD) [13]. This sediment has 11 certified and 24 non-certified concentrations for PAHs at levels of 40–1300 ng/g. SRM 1939, which is certified for PCB content (see below), is a river sediment collected from the Hudson River (NY) and is representative of sediment with high levels of PCB congeners and chlorinated pesticides (100–7000 ng/g

TABLE 12.7

CONCENTRATIONS OF PACs IN SRM 1597, COMPLEX MIXTURE OF PAHs FROM COAL TAR

Peak no. ^a	Compound	Concentration ($\mu\text{g/g}$) ^b
1	Naphthalene	1160 \pm 50
2	Benzothiophene	27.5 (0.6) ^c
3	2-Methylnaphthalene	97.1 (1.1) ^c
4	1-Methylnaphthalene	47.0 (0.6) ^c
5	Biphenyl	27.4 (0.3) ^c
10	Acenaphthylene	252 (1) ^c
14	Dibenzofuran	88.9 (0.5) ^c
19	Fluorene	136 (1) ^c
30	Dibenzothiophene	230 (0.4) ^c
31	Phenanthrene	462 \pm 3
32	Anthracene	101 \pm 2
34	Carbazole	39.0 (0.4) ^c
39	4 <i>H</i> -Cyclopenta[<i>def</i>]phenanthrene	51.3 (0.3) ^c
43	Fluoranthene	322 \pm 4
44	Accephenanthrylene	59.5 (0.9) ^c
46	Pyrene	235 \pm 2
65	4 <i>H</i> -Cyclopenta[<i>cd</i>]pyrene	38.0 (0.6) ^c
66	Benz[<i>a</i>]anthracene	98.6 \pm 3.6
67	Chrysene	71.7 \pm 1.0
67	Triphenylene	12.1 \pm 0.4
75	Benzo[<i>b</i>]fluoranthene	70.1 (4.2) ^d , 61.4 (1.7) ^e
77	Benzo[<i>k</i>]fluoranthene	47.4 (4.1) ^d , 38.8 (1.3) ^e
79	Benzo[<i>e</i>]pyrene	57.1 (0.5) ^c
80	Benzo[<i>a</i>]pyrene	95.8 \pm 5.8
81	Perylene	26.1 \pm 1.0
87	Indeno[1,2,3- <i>cd</i>]pyrene	60.2 \pm 4.4
90	Picene	12.1 (0.7) ^c
91	Benzo[<i>ghi</i>]perylene	53.7 \pm 7.6
92	Anthanthrene	31.8 ^c , 26.7 (3.2) ^e
100	Coronene	11.3 (1.3) ^c

^aPeak number refers to Fig. 12. 2 [23].^bCertified values (in bold) were obtained from the combined results of the GC and LC analyses; the associated uncertainties are \pm two standard deviations of the mean values of the two techniques. Non-certified concentrations in parentheses obtained from one analytical technique; uncertainties are \pm one standard deviation of a single measurement [23].^cGC analysis [23].^dGC analysis on a liquid crystalline stationary phase column [23].^eLC analysis [23].

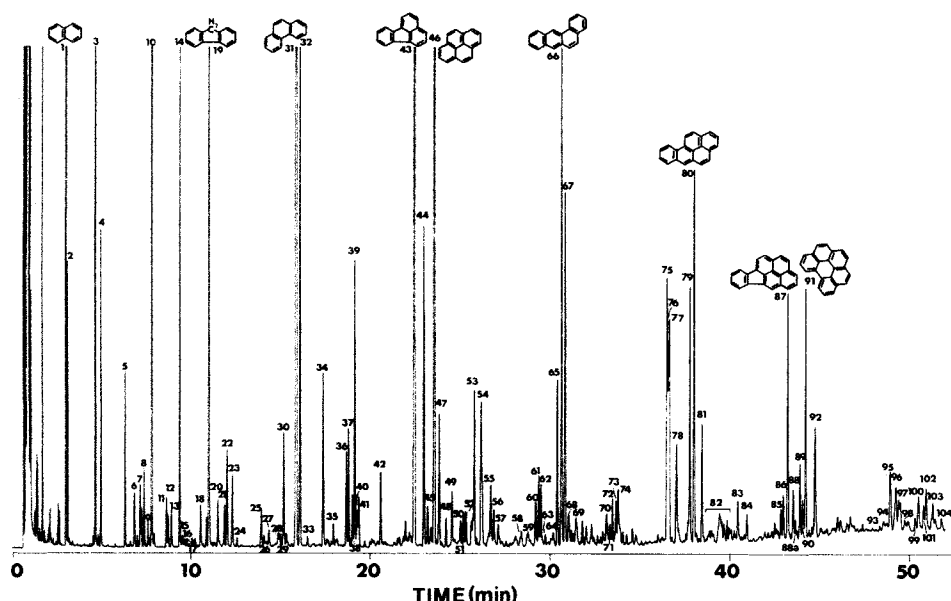


Fig. 12.2. GC-FID analysis of SRM 1597 (Complex Mixture of PAHs from Coal Tar). Column: 30-m capillary column with 5% phenyl-substituted methylpolysiloxane stationary phase. Major peaks identified in Table 12.7; complete identification of peaks in ref. 23.

and 60–550 ng/g, respectively), but low levels of PAHs (50–200 ng/g) [29]. The concentrations of PAHs on SRM 1939 are provided only as non-certified values.

The majority of biological tissue reference materials available from NIST and other producers of reference materials (primarily for trace element determinations) have been distributed as lyophilized (freeze-dried) matrices. In recent years, some limitations of these traditional biological and environmental reference materials have been recognized. The requirements for a reference material regarding physical and/or chemical stability, stable dry weight and sterility have restricted the matrices that could be used as reference materials, or have necessitated sample preparation that often significantly alters the physical or chemical properties of the material. Sample preparation procedures for previous biological marine reference materials have included freeze drying, fat extraction, radiation sterilization or more extreme measures such as cooking. The physical characteristics of these materials are, in many instances, significantly different from the sample matrix actually analyzed (this is particularly true for samples analyzed for trace organic constituents), thus the value of these materials as quality control samples is often limited. To meet the need for a natural matrix marine tissue reference material for organic contaminants, NIST issued SRM 1974 (Organics in Mussel Tissue (*Mytilus edulis*)), in 1990 [14]. Approximately 2500 mussels were collected in Boston Harbor (MA) and the shells removed to provide 30 kg of tissue. This material was then cryogenically homogenized and the SRM is provided as a frozen powder-like homogenate, thereby providing a matrix similar to the sample matrices typically encountered in marine tissue analyses. SRM 1974

has certified concentrations for 9 PAHs and non-certified concentrations for 19 additional PAHs. Even though this material was developed primarily for organic constituents, non-certified concentrations of 36 trace elements are reported, thereby providing a reference material that satisfies the matrix requirements of inorganic analysts who routinely analyze fresh tissue samples.

12.2.2.2. Analytical methods for the determination of PAHs in natural matrix SRMs

At NIST the approach to certification of environmental matrix SRMs is based on the use of two or more “independent” analytical methods. The required independent analytical procedures include different extraction and clean-up/isolation procedures as well as separation and detection techniques. Prior to the certification of the first environmental matrix SRM for organic constituents, SRM 1580 (Organics in Shale Oil), several analytical procedures for the determination of individual PACs in complex matrices were evaluated and the results compared [8]. These analytical methods included clean-up procedures of normal-phase LC, acid-base extraction, or no extraction/isolation step followed by separation and quantification by using LC, GC or GC-MS. Two significant developments in analytical methodology made possible the accurate determination of individual PAHs in this complex oil matrix: (1) direct injection GC-MS analysis with little or no sample clean-up and (2) normal-phase LC on a polar, chemically bonded stationary phase to isolate the PAHs of interest in fractions that were more suitable for analysis by GC, LC and GC-MS [8,35]. These developments provided the necessary two independent analytical methods, both clean-up and separation/quantification procedures, to allow certification of the shale oil SRM and later the petroleum crude oil SRM.

During the development of subsequent organic environmental SRMs, the analytical methods improved to provide greater precision and often greater accuracy in the PAH measurements. One factor contributing to this improvement was the use of perdeuterated PAHs as internal standards for quantification in both LC and GC-MS. For the certification of SRM 1580, several approaches for quantification were used including: internal standards, external standards and the standard addition method [8,9]. For SRMs 1582, 1649 and 1650, perdeuterated PAH were used as internal standards for both LC and GC-MS measurements [10–12]. The relative uncertainties of the certified values for SRM 1580 are generally 15–30%. For SRMs 1582, 1649 and 1650, the uncertainties of the certified values were generally lower at 10–15%. For SRM 1582 in particular, which is a similar oil matrix, the uncertainties of the certified values were between 5 and 10% with one exception (benzo[*a*]pyrene). The use of perdeuterated PAHs as internal standards for the LC analysis of SRM 1582 has been described in detail [11]. During the LC certification measurements of SRM 1582, perylene was re-determined in SRM 1580 to resolve the previous discrepancy between the LC and GC-MS measurements (3.9 ± 0.6 and $2.8 \pm 0.6 \mu\text{g/g}$, respectively). Using this improved LC methodology, which included the use of a C-18 column with different selectivity, a value of $2.5 \pm 0.2 \mu\text{g/g}$ was obtained which is in good agreement with the certification GC-MS measurements. The earlier higher LC value included a contribution from benzo[*k*]fluoranthene which co-eluted with the perylene in the original certification measurements. Several years after the certification of SRM 1580, four additional compounds (phenanthrene, anthracene, benz[*a*]-

anthracene and chrysene) were determined by both LC and GC-MS with good agreement between the two techniques (see Table 12.6) [30,31]. Benzo[ghi]perylene and indeno[1,2,3-cd]pyrene were also determined in SRM 1580 using GC-MS only [30]. Two additional PAHs, benzo[b]fluoranthene and benzo[k]fluoranthene, have been determined by an LC method with quenchofluorometric detection [32]. Thus, a total of 13 PAHs have been reported in SRM 1580 as indicated in Table 12.6.

SRM 1597 consists of a natural mixture of PAHs isolated from a crude coke oven tar and dissolved in toluene. This SRM is intermediate in complexity when compared to the calibration solutions and the natural matrix materials such as oil, sediment and air and diesel particulate material in that it requires no clean-up prior to analysis by GC or LC. This SRM has an extensive summary of PAC concentrations (12 certified and 18 non-certified). The certified and non-certified concentrations for SRM 1597 are provided in Table 12.7. For quantification of the compounds in SRM 1597, GC with flame ionization detection (GC-FID) and reversed-phase LC with fluorescence detection (LC-FL) were used as the two independent analytical techniques. A detailed discussion of the analytical methods used for the certification measurements and a comparison of the results from the various methods have been published [23]. For the majority of the compounds listed in Table 12.7, both the GC-FID and LC-FL analyses were performed without concentration or clean-up of the sample (i.e. direct analysis). The chromatogram from the GC analysis of SRM 1597 is shown in Fig. 12.2. The reversed-phase LC analysis of SRM 1597 is shown in Fig. 12.3. Reversed-phase LC with wavelength programmed fluorescence detection was first developed for the analysis of SRM 1649 [12]. In this procedure, the excitation and emission wavelength conditions are changed during the chromatographic analysis to maximize the sensitivity and/or selectivity of the detection for each analyte. However, by using this direct analysis LC method, it was not possible to obtain accurate data for triphenylene or benzo[ghi]perylene because of low detection sensitivity or selectivity. To obtain LC data for these two compounds, a normal-phase LC fractionation procedure [23,35] was employed to isolate separate fractions containing these components that were suitable for reversed-phase LC analysis. Chrysene and triphenylene are not routinely quantified by GC analyses since they co-elute on conventional non-polar stationary phases. Concentrations for chrysene and triphenylene as determined by GC are generally reported as a combined value. To provide the necessary data from a second technique for certification, these two isomeric PAHs were determined by GC analysis on a liquid crystalline stationary phase [23]. SRM 1597 was the first SRM with certified concentrations for chrysene and triphenylene (see Tables 12.6–12.8).

Approximately 60 compounds were identified in SRM 1597 based on molecular weight information from GC-MS analysis and on the comparison of GC retention data with reference standards and literature data. GC retention indexes, as described by Lee and co-workers [36] were determined for a number of the compounds in this material and are compared with literature data on the Certificate of Analysis. In addition to the PAH constituents, SRM 1597 also contains other PACs as minor components including sulfur heterocycles (e.g. dibenzothiophene, phenanthro[4,5-*bcd*]thiophene, and three benzo-naphthothiophene isomers), nitrogen heterocycles (carbazole and 4*H*-benzo[*def*]carbazole) and oxygen heterocycles (dibenzofuran). The detailed characterization of these minor heterocyclic constituents in SRM 1597 has not been performed; however, Nishioka

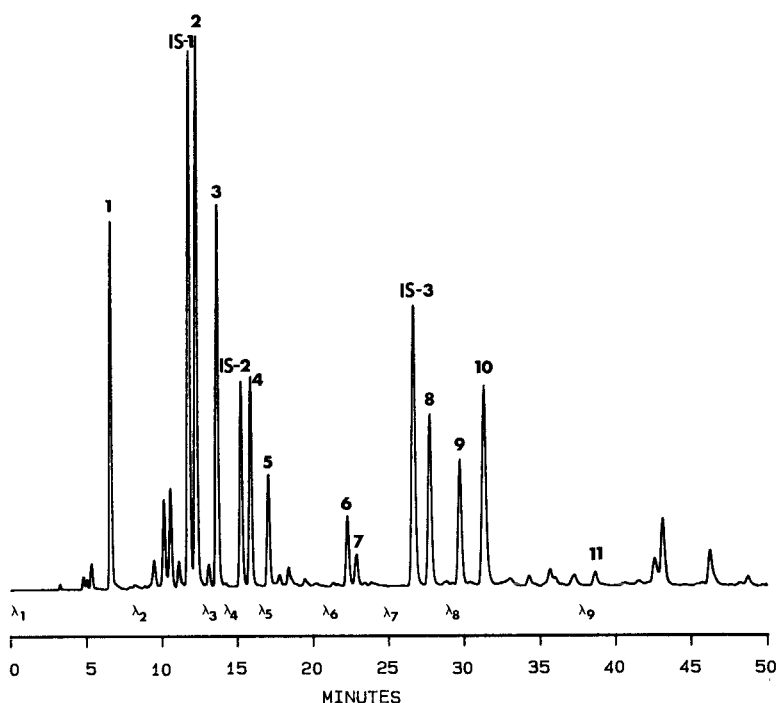


Fig. 12.3. Reversed-phase LC analysis of SRM 1597 using wavelength programmed fluorescence detection. Column: C-18 column (Vydac 201TP), 5- μ m particle size, 4.6 mm i.d. \times 25 cm. Mobile phase conditions: linear gradient from 50% acetonitrile in water to 100% acetonitrile in 50 min. Fluorescence conditions (excitation λ /emission λ in nm): λ_1 (280/340), λ_2 (249/380), λ_3 (250/442), λ_4 (285/450), λ_5 (333/390), λ_6 (285/385), λ_7 (406/440), λ_8 (296/405) and λ_9 (300/500). Peak identifications: (1) naphthalene, (IS-1) phenanthrene- d_{10} , (2) phenanthrene, (3) anthracene, (IS-2) fluoranthene- d_{10} , (4) fluoranthene, (5) pyrene, (6) benz[a]anthracene, (7) chrysene, (IS-3) perylene- d_{12} , (8) perylene, (9) benzo[k]fluoranthene, (10) benzo[a]pyrene, (11) indeno[1,2,3-*cd*]pyrene (see ref. 23).

et al. [37–39] have analyzed the original coal tar material used in the preparation of this SRM and have reported extensive qualitative and quantitative information on PAHs, sulfur heterocycles, nitrogen-containing polycyclic aromatic compounds, oxygen-containing compounds and nitrogen-containing thiophenic compounds. Thirteen PAH isomers of molecular weight 302 have also been identified in SRM 1597 [40]. PAH mixtures from coal tar have already found use in a number of laboratories for the evaluation of gas chromatographic performance (e.g. column efficiency and/or selectivity) [41–45]. Therefore, SRM 1597 serves as a common reference sample for such comparisons among various laboratories.

In 1989 and 1990, the marine sediment and mussel tissue SRMs were certified using the general approach developed and refined during the certification of previous SRMs for PAHs. Using the experience gained from the previous SRMs, a larger number of analytes

TABLE 12.8

CONCENTRATIONS OF PAHs IN NATURAL MATRIX SRMs (SEDIMENTS AND MUSSEL TISSUE)^a

Compound	River sediment SRM 1939 ($\mu\text{g/g}$) ^b	Marine sediment SRM 1941 ($\mu\text{g/g}$) ^c	Mussel tissue SRM 1974 (ng/g dry wt) ^d
Naphthalene		1.322 (0.014)	
2-Methylnaphthalene		0.406 (0.036)	17 (4)
1-Methylnaphthalene		0.229 (0.019)	9 (2)
Biphenyl		0.115 (0.015)	
Acenaphthylene		0.115 (0.010)	
Acenaphthene		0.052 (0.002)	
Fluorene		0.104 (0.005)	12 (2)
Phenanthrene	0.13 (0.01)	0.577 \pm 0.059	45 \pm 11
Anthracene		0.202 \pm 0.042	6.1 \pm 1.7
3-Methylphenanthrene		0.150 (0.005)	
2-Methylphenanthrene		0.190 (0.006)	
2-Methylanthracene		0.066 (0.007)	
9-Methyl/4-methylphenanthrene		0.145 (0.008)	22 (5)
1-Methylphenanthrene		0.109 (0.006)	19 (5)
Fluoranthene	0.19 (0.01)	1.22 \pm 0.24	272 \pm 47
Pyrene	0.17 (0.02)	1.08 \pm 0.20	276 \pm 30
Chrysene	0.051 ^e (0.001)	0.449	124 ^f (11)
Triphenylene		0.192 (0.003)	
Benz[a]anthracene	0.046 (0.001)	0.550 \pm 0.078	37 (3)
Benzo[a]fluoranthene		0.146 (0.004)	4.1 (1.2)
Benzo[b]fluoranthene		0.78 \pm 0.19	52.3 \pm 9.4
Benzo[j]fluoranthene		0.351 (0.014)	
Benzo[k]fluoranthene		0.444 \pm 0.049	24 (1)
Benzo[e]pyrene		0.573	81 (6)
Benzo[a]pyrene		0.67 \pm 0.13	18.6 \pm 3.8
Perylene		0.422 \pm 0.033	8.5 \pm 2.4
Indeno[1,2,3- <i>cd</i>]pyrene		0.569 \pm 0.040	14.6 \pm 2.7
Benzo[ghi]perylene		0.516 \pm 0.083	20.0 \pm 2.3

^aCertified concentrations in bold; see Certificate of Analysis for each SRM. Certified concentrations were obtained from the combined results of at least two independent analytical methods and the uncertainties are generally a 95% prediction interval with an allowance for systematic error among the methods used. Non-certified values are generally results from one analytical technique and the uncertainties (values in parentheses) are one standard deviation of a single measurement assuming all measurements are statistically independent and identically distributed.

^bSee ref. 29. ^cSee ref. 13. ^dSee ref. 14.

^eConcentration includes both chrysene and triphenylene.

(9–11 certified and 19–24 non-certified PAHs) was measured in these two marine matrix materials than the earlier SRMs. The general analytical approach for the certification of PAHs in environmental SRMs is illustrated in Fig. 12.4 for the certification of SRM 1941 (Organics in Marine Sediment). Three analytical techniques were used in the measurement of PAHs in SRM 1941: GC-FID; GC-MS; reversed-phase LC-FL. These three techniques have been used extensively at NIST for the measurement of PAHs in environmental samples [6]. Recently, the results of GC-MS and LC-FL analyses of several SRMs and reference samples were summarized and the comparability of the two techniques was discussed in detail [34]. At present GC-MS and LC-FL are the two primary techniques used at NIST for the measurement of PAHs in natural matrix SRMs.

For each of the three approaches used for the determination of PAHs in SRM 1941 (see Fig. 12.4), different extraction solvents and preparation steps were employed, as well as several different internal standards. Sample preparation for both the GC-FID and LC-FL analyses involved the isolation of the PAH fraction using normal-phase LC. However, the degree of clean-up of the PAH fraction was dependent on the selectivity of the final detection step. Since the flame ionization detector is a universal detector, the PAH fraction was isolated from the aliphatic hydrocarbons and the more polar compounds by using normal-phase LC on an aminosilane column. However, since fluorescence detection in LC provides excellent selectivity for the determination of PAHs, a relatively simple clean-up on a short solid phase extraction column (aminosilane) could be used to remove only the more polar compounds. Mass spectrometry offers the most selective detection approach, provided that the PAH isomers are separated from each other by GC. Because of the selectivity of MS detection, the extracts were analyzed directly by GC-MS with no clean-up or PAH isolation step, thereby providing an approach completely independent of the LC clean-up steps used by both the GC-FID and LC-FL approaches.

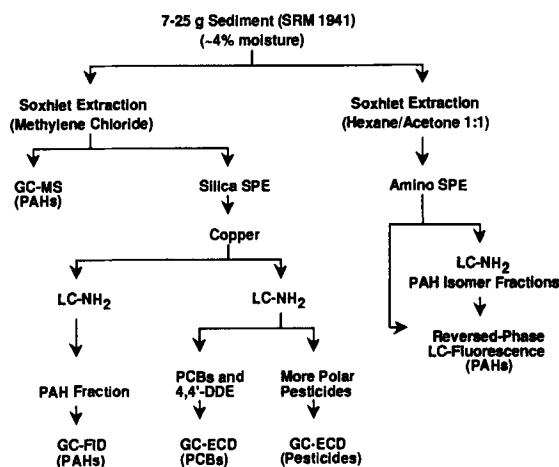


Fig. 12.4. Analytical scheme for the determination of organic constituents in SRM 1941 (Organics in Marine Sediment) (see ref. 13).

TABLE 12.9

SUMMARY OF ANALYTICAL RESULTS (ng/g DRY WEIGHT) FOR THE DETERMINATION OF PAHs IN SRM 1941, ORGANICS IN MARINE SEDIMENT

Compound	GC-FID	GC-MS	LC-FL (direct)	LC-FL (fraction)
Phenanthrene	597 (4) ^b	603 (10)	531 (12)	
Anthracene	202 (6)	228 (12)	174 (8)	
Fluoranthene	1116 (20)	1401 (41)	1135 (10)	
Pyrene	1008 (16)	1238 (18)	989 (34)	
Benz[<i>a</i>]anthracene	538 (12)	599 (14)	516 (7)	521 (11) ^d
Chrysene	577 (12) ^c	702 (16) ^c	425 (42)	473 (5) ^d
Triphenylene				192 (3) ^d
Benzo[<i>b</i>]fluoranthene	635 (17)	864 (28)	839 (14)	843
Benzo[<i>j</i>]fluoranthene	351 (14)			
Benzo[<i>k</i>]fluoranthene	439 (19)	857 (25) ^f	456 (6) ^e 441 (8) ^e	443 (16)
Benzo[<i>e</i>]pyrene	472 (25)	672 (24)		
Benzo[<i>a</i>]pyrene	566 (12)	754 (49)	674 (12)	690 (25)
Perylene	415 (8)	437 (27)	411 (6)	426 (5)
Benzo[<i>ghi</i>]perylene	478 (14)	566 (26)		504 (7)
Indeno[1,2,3- <i>cd</i>]pyrene	572 (28)	559 (19)	573 (20)	575 (8)

^aConcentrations reported on dry weight basis; material as received contains residual moisture.^bUncertainties (values in parentheses) for GC-FID, LC-FL and GC-MS measurements are \pm one standard deviation of a single measurement; for GC-FID measurements, 12 samples analyzed in triplicate; for LC measurements, three samples analyzed in triplicate; for GC-MS measurements, four samples analyzed in duplicate.^cValue is for chrysene and triphenylene.^dDetermined using triphenylene-*d*₁₂ as internal standard.^eBenzo[*k*]fluoranthene was determined at different times, i.e. during initial analyses of total PAH fraction and during benzo[*b*]fluoranthene analyses.^fValue is for benzo[*k*]fluoranthene and benzo[*j*]fluoranthene.

The results for the determination of 15 major PAHs in SRM 1941 using GC-FID, LC-FL and GC-MS are summarized and compared in Table 12.9. Twelve of the PAHs determined by LC-FL were measured by analysis of the total PAH fraction from the extract (LC-FL (direct) in Table 12.9). However, it was not possible to measure accurately several PAHs in the total PAH fraction (i.e. triphenylene and benzo[*ghi*]perylene) due to their low fluorescence sensitivity and/or interferences as mentioned previously for the coal tar SRM. To provide results for triphenylene and benzo[*ghi*]perylene, a normal-phase LC procedure was used to isolate isomeric PAH fractions which were then analyzed by LC-FL (LC-FL (fraction) in Table 12.9). In the analysis of the total PAH fraction, the peaks for chrysene and benz[*a*]anthracene were not completely resolved from other peaks.

Thus, chrysene and benz[*a*]anthracene were measured along with triphenylene during the analysis of the four ring aromatic fraction. The five aromatic ring fraction was analyzed to determine additional results for perylene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene. The six aromatic ring fraction was analyzed to obtain results for benzo[*ghi*]perylene and additional data for indeno[1,2,3-*cd*]pyrene.

As shown in Table 12.9, the differences among the results from the three techniques generally ranged between 20 and 30% for most of the PAHs. Much better agreement for the results from the three techniques was obtained for phenanthrene, perylene and indeno[1,2,3-*cd*]pyrene, which differed by only 14, 6 and 3%, respectively. In general, the agreement between the LC-FL and the GC-FID results was good with differences of only 1–5% for most of the analytes. The largest difference between the LC-FL and GC-FID results was 18% for benzo[*a*]pyrene.

To measure triphenylene in the sediment, the four aromatic ring fraction was isolated from the total PAH fraction using normal-phase LC and then analyzed by reversed-phase LC as described above for the coal tar SRM [13,23,35]. The two LC results for chrysene (direct and fraction) are in good agreement (425 ± 42 and 473 ± 5 ng/g). The GC-FID and GC-MS results for chrysene/triphenylene are 577 ± 12 and 702 ± 16 , respectively (mean of 639 ng/g) compared to the sum of LC results for triphenylene (192 ng/g) and chrysene (mean of 449 ng/g) which is 641 ng/g.

Six PAHs with five aromatic rings were measured using various combinations of the three analytical techniques. Three benzo[*fluoranthene*] isomers (*b*, *j* and *k*) are difficult to separate using conventional non-polar GC stationary phases. Sufficient resolution was obtained in the GC-FID analyses on the 60-m column to provide results for each isomer. However, in the GC-MS analyses using a similar column, the benzo[*k*]fluoranthene and the benzo[*j*]fluoranthene were not resolved. The agreement among the LC and GC-FID results for benzo[*k*]fluoranthene was excellent, and if the GC-FID results for benzo[*j*]fluoranthene and benzo[*k*]fluoranthene are combined (790 ng/g), the result compares favorably with the GC-MS result for these two PAHs (857 ± 25). For benzo[*b*]fluoranthene, the GC-FID results were about 25% lower than those obtained from the other techniques. Among the three techniques, the results for benzo[*a*]pyrene differed by the largest amount (33%), whereas the results for perylene differed by only 6%.

The largest molecular weight PAHs determined by two or more techniques were benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene. Benzo[*ghi*]perylene is difficult to measure by LC-FL in a total PAH fraction due to co-eluting peaks and low sensitivity and selectivity [34]. Thus, the benzo[*ghi*]perylene fraction (six aromatic ring fraction) was isolated by normal-phase LC to enrich the concentration of the analytes of interest and to reduce the number of potential interfering compounds in the analysis [12]. Using this approach, the LC results for benzo[*ghi*]perylene (504 ± 7 ng/g) were in good agreement with the GC-FID results (478 ± 14 ng/g) and within about 12% of the GC-MS measurements (567 ± 26 ng/g). For indeno[1,2,3-*cd*]pyrene, all four results agreed within 3%, i.e. 572 ± 28 ng/g (GC-FID), 573 ± 20 ng/g (LC direct), 575 ± 8 ng/g (LC fraction) and 559 ± 19 ng/g (GC-MS).

Based on the results from the four different analytical approaches summarized in Table 12.9, certified concentrations of 11 PAHs were determined and these values are provided in Table 12.8. In addition to the 15 PAHs listed in Table 12.9, 24 additional PAHs, in-

cluding methyl- and dimethylphenanthrene/anthracene isomers, were determined by the GC-MS procedure to provide non-certified values which are also included in Table 12.8.

A similar analytical approach using only GC-MS and LC-FL was used in the certification of SRM 1974 (Organics in Mussel Tissue (*Mytilus edulis*)) [14]. The analytical results for the determination of nine PAHs using both LC-FL and GC-MS, are summarized and compared in Table 12.10. Again, there is generally good agreement between the LC-FL and GC-MS determinations. Differences in the mean values for the two techniques were 1–4% for phenanthrene, anthracene, perylene and benzo[ghi]perylene; 12% for fluoranthene and pyrene; and 13–15% for benzo[a]pyrene, benzo[b]fluoranthene and indeno[1,2,3-*cd*]pyrene. Based on the results from these two techniques, certified concentrations of these nine PAHs were determined and are summarized in Table 12.8. The concentrations for 19 additional PAHs were also measured using only one analytical technique (either GC-MS or LC-FL) and are provided in the Certificate of Analysis as non-certified values. Concentrations for nine of these PAHs are provided in Table 12.8.

The general approach for the certification of PAHs in future environmental SRMs will include LC-FL and GC-MS on two columns of different selectivity (a non-polar phase and a liquid crystalline phase). This approach is being used to certify the new marine sediment and tissue SRMs and for the recertification of SRMs 1649 and 1650 (see discussion below).

TABLE 12.10

SUMMARY OF ANALYTICAL RESULTS (ng/g DRY WEIGHT) FOR THE DETERMINATION OF PAHs IN SRM 1974, ORGANICS IN MUSSEL TISSUE (*MYTILUS EDULIS*)

Compound	LC-FL ^a	GC-MS ^a	Certified ^b
Phenanthrene	44.6 (2.7)	45.3 (7.3)	45 ± 11
Anthracene	5.97 (0.52)	6.14 (0.72)	6.1 ± 1.7
Fluoranthene	289 (10)	255 (21)	272 ± 47
Pyrene	294 (10)	259 (12)	276 ± 30
Perylene	8.56 (0.35)	8.5 (1.7)	8.5 ± 2.4
Benzo[b]fluoranthene	55.9 (2.2)	48.7 (5.2)	52.3 ± 9.4
Benzo[a]pyrene	20.1 (2.3)	17.1 (2.2)	18.6 ± 3.8
Benzo[ghi]perylene	19.6 (1.4)	20.3 (2.3)	20.0 ± 2.3
Indeno[1,2,3- <i>cd</i>]pyrene	15.6 (1.4)	13.6 (1.4)	14.6 ± 2.7

^aUncertainties in parenthesis are one standard deviation of a single measurement treating all measurements as statistically independent and identically distributed; LC-FL results are from analyses of six samples and GC-MS results are from analyses of 12 samples.

^bThe certified values are equally weighted means of results from two analytical techniques. The uncertainty is obtained from a 95% prediction interval plus an allowance for systematic error between the methods used. In the absence of systematic error, the resulting uncertainty limits will cover the concentration of approximately 95% of samples of this SRM having a minimum sample size of 15 g (wet weight). Certified values were determined on a wet weight basis; concentrations were converted to a dry weight basis for user convenience.

TABLE 12.11

CONCENTRATIONS ($\mu\text{g/g}$) OF ADDITIONAL PAHs IN AIR AND DIESEL PARTICULATE SRMs^a

Compounds	Air particulate matter SRM 1648	Urban dust/ organics SRM 1649	Diesel particulate matter SRM 1650
3-Methylphenanthrene		0.65 (0.03) ^b	77.8 (9.8) ^b
2-Methylphenanthrene	0.99 ^c	0.94 (0.04) ^b 0.80 ^c	101 (14) ^b
Benzo[ghi]fluoranthene	1.44 ^c	1.3 (0.3) ^b	20.4 (3.1) ^b
Acephenanthrylene	1.8 (0.2) ^d		9.5 (0.9) ^d
8-Methylfluoranthene	1.1 (0.1) ^d		7.1 (0.6) ^d
2-Methylpyrene	0.5 (0.1) ^d		5.4 (0.5) ^d
4-Methylpyrene	0.5 (0.1) ^d		5.6 (0.6) ^d
1-Methylpyrene	0.3 (0.1) ^d		2.5 (0.3) ^d
Chrysene/triphenylene	7.8 (0.6) ^c	4.4 (0.4) ^b	29.0 (3.6) ^b
Triphenylene	2.6 (0.1) ^d	1.7 (0.1) ^e	
Benzo[fluoranthenes [b,j, and k]	16.0 (1.1) ^d	9.1 (1.4) ^b	10 (1) ^f
Coronene		3.7 (1.2) ^b	

^aUncertainties (values in parentheses) are one standard deviation of the mean value of replicate analyses, generally 3–4 samples.

^bGC analysis [33]. ^cGC-MS [25]. ^dGC analysis [24]. ^eLC-FL [12]. ^fGC-MS [48].

12.2.2.3. Measurements of additional PAHs

In addition to the PAHs reported in Tables 12.6–12.8, additional PAHs and related compounds have been measured in these natural matrix SRMs after the materials have been issued as SRMs. For example, Table 12.11 summarizes results for a number of additional PAHs measured in the three particulate SRMs as part of analytical methods development studies. The three particulate material SRMs (1648, 1649 and 1650) have been characterized and compared in several reports [24,25].

Recently the concentrations of PAH isomers of molecular weight 278 (dibenzanthracene isomers) and 302 (dibenzopyrene/fluoranthene isomers) were measured in four of the natural matrix SRMs as part of the development of LC methods for these isomers [46]. The measurement of individual PAHs in both of these groups of isomers has been limited for several reasons: (1) they are generally present in environmental samples at levels 5–20 times lower than PAHs of similar molecular weight that are routinely measured (e.g. benzo[a]pyrene or benzo[ghi]perylene); (2) both of these isomer groups contain a large number of possible isomers (i.e. 12 possible isomers of MW 278 and 33 possible isomers of MW 302); (3) many of the isomers are not sufficiently resolved on conventional GC and LC columns; (4) reference standards of many of the isomers are not readily available. However, in spite of these difficulties, the measurement of isomers in these two

groups could provide valuable information since the carcinogenicity of the different isomers varies significantly and the relative amounts of the various isomers may be indicative of the source of the PAHs. These isomers were measured using a multi-dimensional LC procedure consisting of normal-phase LC to isolate the PAH isomer groups followed by analysis of the isomeric groups by reversed-phase LC with fluorescence detection. Using the multidimensional LC procedure, six isomers of MW 278 and nine isomers of MW 302 were quantified in SRMs 1597, 1648, 1649 and 1941 and the results are summarized in Tables 12.12 and 12.13.

12.2.2.4. Measurements of aliphatic hydrocarbons

Recently, the concentrations of aliphatic hydrocarbons were determined in the two marine matrix SRMs, SRMs 1941 and 1974, and the results are summarized in Table 12.14 [47]. These results, which are not provided on the Certificate of Analysis are not certified and are only provided as additional information, may be useful for measurements associated with assessing petroleum contamination in the marine environment.

12.2.2.5. Measurements of nitro-PAHs and other PACs

Nitro-PAHs have also been measured in the two air particulate materials and diesel particulate material and the data are summarized in Table 12.15. The nitro-PAHs have been determined by LC-fluorescence after reduction to the amino-substituted PAHs [26], LC with electrochemical detection [26] and GC-MS [48]. A comparison of these three techniques for the measurement of nitro-PAH has been reported by MacCrehan et al. [26]. For the two oil matrix SRMs, certified and non-certified values are provided for nine phenols [19,21], three *N*-heterocycles [20] and dibenzothiophene [22] (see Table 12.16).

TABLE 12.12

CONCENTRATIONS (ng/g) OF SELECTED PAH ISOMERS OF MW 278 IN SEVERAL ENVIRONMENTAL SRMs^a

Compound	Marine sediment (Baltimore, MD) SRM 1941	Air particulate matter (St. Louis, MO) SRM 1648	Air particulate matter (Washington, DC) SRM 1649	Coal tar SRM 1597
Dibenz[<i>a,c</i>]anthracene	64.3 (2.5)	261 (12)	190 (14)	4540 (130)
Dibenz[<i>a,j</i>]anthracene	98.0 (4.7)	328 (9)	266 (14)	6820 (270)
Pentaphene	57.0 (1.4)	158 (8)	148 (4)	5050 (400)
Dibenz[<i>a,h</i>]anthracene	88.9 (5.6)	275 (9)	221 (10)	6760 (510)
Benzo[<i>b</i>]chrysene	103 (6)	246 (3)	258 (28)	10300 (430)
Picene	121 (8)	518 (14)	386 (39)	6750 (300)

^aUncertainties (values in parentheses) are \pm one standard deviation of a single measurement.

TABLE 12.13

CONCENTRATIONS (ng/g) OF SELECTED PAH ISOMERS OF MW 302 IN SEVERAL ENVIRONMENTAL SRMs^a

Compound	Marine sediment (Baltimore, MD) SRM 1941	Air particulate matter (St. Louis, MO) SRM 1648	Air particulate matter (Washington, DC) SRM 1649	Coal tar SRM 1597
Naphtho[2,3- <i>e</i>]pyrene	64 (2)	289 (23)	235 (16)	4870 (180)
Dibenzo[<i>a,e</i>]pyrene	135 (16)	691 (39)	625 (30)	9730 (270)
Naphtho[1,2- <i>k</i>]fluoranthene	124 (6)	717 (64)	542 (36)	10150 (210)
Dibenzo[<i>b,k</i>]fluoranthene	150 (9)	938 (150)	795 (64)	10540 (100)
Naphtho[2,3- <i>b</i>]fluoranthene	53 (3)	283 (23)	224 (11)	3770 (130)
Dibenzo[<i>a,i</i>]pyrene	32 (1)	121 (3)	125 (7)	3400 (70)
Naphtho[2,3- <i>a</i>]pyrene	14.0 (0.2)	52 (4)	56 (3)	3510 (100)
Naphtho[2,3- <i>k</i>]fluoranthene	12.7 (0.2)	49 (3)	56 (2)	2030 (80)
Dibenzo[<i>a,h</i>]pyrene	15 (1)	52 (2)	52 (1)	1940 (160)

^aUncertainties (values in parentheses) are \pm one standard deviation of a single measurement.

12.2.2.6. Reference values for mutagenic activity

Because of the interest in the mutagenic activity of complex mixtures of PAHs and other organic analytes extracted from environmental samples, reference values for Ames bioassay mutagenicity have been determined for three NIST SRMs: SRM 1597 (Complex Mixture of PAHs from Coal Tar); SRM 1649 (Urban Dust/Organics); SRM 1650 (Diesel Particulate Matter). The mutagenicity reference values were determined as part of an international collaborative study, sponsored by the International Programme on Chemical Safety (IPCS) and the US EPA, to study the mutagenicity of complex environmental mixtures in the Ames *Salmonella typhimurium* mutation assay [49]. Samples of SRM 1597, SRM 1649 and SRM 1650 were assayed by 20 laboratories and the results compiled to determine the reference mutagenicity values summarized in Table 12.17. A detailed discussion of the treatment of the data to establish the reference values and the use of these reference values has been reported by Claxton et al. [50]. The mean mutagenic activity based on the results from the 20 participating laboratories (see Table 12.17) provides an estimate of the bacterial mutagenic potency of the extracts from the three SRMs [50]. The data from the collaborative study have been used to establish two types of reference values. The first value, expressed as a 95% confidence interval, defines the actual mutagenic activity as measured by the Ames bioassay test. The second type of reference values, expressed as an 80% tolerance interval, characterizes the differences in reported mutagenic activity and establishes a realistic target for comparing results from the Ames bioassay with state-of-the-practice. With these Ames mutagenicity reference values, SRMs 1597, 1649 and 1650 can now be used by the international bioassay measurement community as

powerful tools for reducing the variability of Ames bioassay data. The mutagenic activity of the diesel particulate material (SRM 1650) is greater than that of the air particulate material (SRM 1649) by about a factor of 10–40. The mutagenic potencies of the two particulate SRMs are not comparable to the coal tar extract because of the different units of measurement (rev/mg versus rev/ μ l) [50]. A special issue of the journal *Mutation Research* provides a thorough discussion of the results of the IPCS Collaborative Study on Complex Mixtures and other related studies using these three SRMs [52].

12.2.3. Natural matrix SRMs for PCB and pesticide determination

The first natural matrix SRMs for PCB measurements were oil and human serum matrices that were fortified with known quantities of Aroclor mixtures. SRM 1581

TABLE 12.14

CONCENTRATIONS (μ g/kg DRY WEIGHT) OF ALIPHATIC HYDROCARBONS IN SRMs 1974 AND 1941

Compound	Mussel tissue SRM 1974 ^a	Marine sediment SRM 1941 ^b
<i>n</i> -Decane (<i>n</i> -C ₁₀)	337 (30)	<1
<i>n</i> -Undecane (<i>n</i> -C ₁₁)	555 (34)	<1
<i>n</i> -Dodecane (<i>n</i> -C ₁₂)	164 (12)	<1
<i>n</i> -Tridecane (<i>n</i> -C ₁₃)	184 (11)	25 (2)
<i>n</i> -Tetradecane (<i>n</i> -C ₁₄)	160 (11)	35 (2)
<i>n</i> -Pentadecane (<i>n</i> -C ₁₅)	288 (15)	93 (5)
<i>n</i> -Hexadecane (<i>n</i> -C ₁₆)	221 (14)	71 (3)
<i>n</i> -Heptadecane (<i>n</i> -C ₁₇)	647 (36)	44 (2)
Pristane	239 (17)	<1
<i>n</i> -Octadecane (<i>n</i> -C ₁₈)	105 (6)	96 (3)
Phytane	191 (15)	<1
<i>n</i> -Nonadecane (<i>n</i> -C ₁₉)	66 (4)	136 (5)
<i>n</i> -Eicosane (<i>n</i> -C ₂₀)	53 (3)	114 (5)
<i>n</i> -Docosane (<i>n</i> -C ₂₂)	61 (4)	140 (6)
<i>n</i> -Tetracosane (<i>n</i> -C ₂₄)	87 (4)	199 (8)
<i>n</i> -Hexacosane (<i>n</i> -C ₂₆)	63 (4)	194 (8)
<i>n</i> -Octacosane (<i>n</i> -C ₂₈)	57 (2)	226 (8)
<i>n</i> -Triacontane (<i>n</i> -C ₃₀)	52 (4)	157 (6)
<i>n</i> -Dotriacontane (<i>n</i> -C ₃₂)	64 (3)	132 (5)
<i>n</i> -Tetratriacontane (<i>n</i> -C ₃₄)	31 (2)	<1

^aResults reported on dry weight basis; four samples extracted and analyzed in duplicate. The uncertainties (values in parentheses) are one standard deviation of a single measurement.

^bResults reported on a dry weight basis; the material contains approximately 4% residual moisture. Two samples extracted and analyzed in duplicate. The uncertainties (values in parentheses) are one standard deviation of a single measurement.

TABLE 12.15

CONCENTRATIONS ($\mu\text{g/g}$) OF NITRO-PAHs IN AIR AND DIESEL PARTICULATE MATTER^a

Compounds	Air particulate matter SRM 1648	Urban dust organics SRM 1649	Diesel particulate matter SRM 1650
2-Nitrofluorene			0.27 ^b
9-Nitroanthracene	0.41 (0.03) ^{b,e}		10 \pm 2 ^b
1-Nitropyrene	0.25 (0.01) ^{c,e} 0.085 (0.005) ^{b,e} 0.39 (0.05) ^{d,e}	0.057 (0.002) ^c	19 \pm 2
7-Nitrobenz[<i>a</i>]anthracene			2.8 ^b
6-Nitrobenzo[<i>a</i>]pyrene			1.6 ^b

^aCertified value in bold; uncertainties (values in parentheses) are \pm one standard deviation of the mean value of replicate analyses, generally 3–4 samples. ^bLC-fluorescence after reduction to amine [26]. ^cLC-EC [26]. ^dGC-MS [48]. ^eConcentration data from [26] converted to $\mu\text{g/g}$; see [48].

TABLE 12.16

CONCENTRATIONS ($\mu\text{g/g}$) OF PACs AND PHENOLS IN SRMs 1580 AND 1582^a

Compound	Shale oil SRM 1580	Petroleum crude oil SRM 1582
Benzo[<i>f</i>]quinoline	16 \pm 4	
Phenanthridine	45 ^b	
Dibenzothiophene		33 \pm 2
Carbazole		3.4 (0.3) ^c
Phenol	407 \pm 50 , 375 (15) ^d	0.26 (0.02) ^d , 0.26 ^e
<i>o</i> -Cresol	385 \pm 50 , 391 (5) ^d	0.42 (0.06) ^d , 0.53 ^e
<i>p</i> -Cresol	273 (7) ^e	
<i>m</i> -Cresol	327 (10) ^e	
2,6-Dimethylphenol	175 \pm 30	
2,5-Dimethylphenol	320 (12) ^e	
2,4-Dimethylphenol	387 (17) ^e	
2,5,6-Trimethylphenol	360 ^f	
2,4,6-Trimethylphenol	120 ^f	
1-Naphthol	19.7 (6.1) ^d	
2-Naphthol	18.9 (3.2) ^d	

^aCertified value in bold; uncertainties (values in parentheses) are \pm one standard deviation of the mean value of replicate analyses, generally 3–4 samples. ^bLC analysis, non-certified value from Certificate of Analysis. ^cGC-MS, positive ion chemical ionization. ^dLC-EC [26].

^eGC [19]. ^fGC-FID, non-certified value from Certificate of Analysis.

TABLE 12.17

REFERENCE VALUES FOR AMES BIOASSAY MUTAGENIC ACTIVITY OF THREE ENVIRONMENTAL MATRIX SRMs^a

Sample	Strain/ activation	Mutagenic activity ^b	95% confidence limits	80% tolerance limits	
				Multiple extraction/ bioassay ^d	Single extraction/ bioassay ^e
SRM 1649	TA100, +S9	102 rev/mg	66–158	30–351	29–365
Urban dust/ organics ^f	TA100, –S9	103 rev/mg	73–146	39–275	36–295
(revertants/mg)	TA98, +S9	214 rev/mg	153–299	83–555	80–570
	TA98, –S9	237 rev/mg	186–301	119–471	115–488
SRM 1650	TA100, +S9	4585 rev/mg	2854–7365	1208–17402	1177–17858
Diesel particulate matter ^f	TA100, –S9	3766 rev/mg	2736–5182	1516–9351	1460–9711
(revertants/mg)	TA98, +S9	2265 rev/mg	1484–3456	679–7550	668–7675
	TA98, –S9	2794 rev/mg	2066–3780	1183–6599	1166–6698
SRM 1597	TA100, +S9	144 rev/ μ l	100–208	51–411	50–416
Complex mixture of PAH from coal tar (revertants/ μ l)	TA98, +S9	60 rev/ μ l	46–79	28–132	26–137

^aResults summarized from ref. 50.^bGeometric mean of all replicate mutagenicity potency values reported by participating laboratories after deleting outlying observations.^cCalculated on a logarithmic scale taking into account both inter- and intra-laboratory variation, excluding outliers, and then re-expressed in the original scale by taking antilogs.^dTolerance limits for mutagenic activity determined in a single laboratory using the same number of replicate extracts/bioassays as in the IPCS collaborative trial.^eTolerance limits for mutagenic activity determined in a single laboratory using only one replicate extraction/bioassay.^fTotal extractable mass (%) with methylene chloride from the IPCS study was 5.0 ± 0.4 for SRM 1649 and 17.5 ± 1.5 for SRM 1650.

(Polychlorinated Biphenyls in Oil), issued in 1982, was prepared by adding 100 μ g/g of Aroclor 1242 and Aroclor 1260 to motor oil and transformer oil matrices. SRM 1589 (Polychlorinated Biphenyls (as Aroclor 1260) in Human Serum), issued in 1985 was prepared in a similar manner with the addition of 106 ng/g of Aroclor 1260. The serum matrix was also fortified with 0.153 ng/g of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin and 0.081 ng/g of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Recently, four marine matrix SRMs have been issued with certified and/or non-certified concentrations of natural levels (i.e. not added) of individual PCB congeners and chlorinated pesticides, i.e. SRM 1588

(Organics in Cod Liver Oil), SRM 1939 (Chlorinated Biphenyls in River Sediment), SRM 1941 (Organics in Marine Sediment) and SRM 1974 (Organics in Mussel Tissue). The concentrations of selected PCB congeners and chlorinated pesticides in these four SRMs are summarized in Tables 12.18 and 12.19, respectively.

The first of these materials, SRM 1588 (Organics in Cod Liver Oil), serves as an excellent surrogate for a tissue extract with a high lipid content. This SRM has certified concentrations for five PCB congeners and ten chlorinated pesticides. However, recently measurements for 20 additional PCB congeners and four additional chlorinated pesticides have been reported to make this SRM the most extensively characterized natural matrix material available with respect to PCB congener and chlorinated pesticide content [28]. The cod liver oil was also fortified with seven polychlorodibenzo-*p*-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs) at the 0.3–1 ng/g level.

SRM 1588 was followed by the two sediment matrices and a mussel tissue described above for PAH measurements, i.e. SRM 1939 (Chlorinated Biphenyl Congeners in River Sediment), SRM 1941 (Organics in Marine Sediment) and SRM 1974 (Organics in Mussel Tissue (*Mytilus edulis*)). SRM 1939 was prepared specifically for PCB congener measurements and is representative of a highly contaminated sediment. SRM 1939 has certified and non-certified concentrations of 17 PCB congeners and 5 pesticides. SRMs 1941 and 1974 were certified for PAHs concentrations, but have non-certified concentrations for PCB congeners and chlorinated pesticides. The concentrations of selected PCB congeners and chlorinated pesticides for the sediment and tissue SRMs are summarized and compared in Tables 12.18 and 12.19.

SRMs 1941 and 1974 are currently the most extensively characterized natural matrix SRMs issued by NIST with certified and non-certified concentrations for a wide variety of environmental contaminants including PAHs, PCBs, pesticides, aliphatic hydrocarbons and trace elements [13,14].

12.2.3.1. Analytical methods for determination of PCB congeners and chlorinated pesticides

The certification of PCB congeners and chlorinated pesticides in natural matrix SRMs requires the same general approach of at least two independent analytical techniques for the measurements. However, in contrast to using both LC and GC for PAHs measurements, GC is the only suitable chromatographic technique at present for these measurements because of the low levels of PCBs and pesticides in environmental matrices and the selectivity and sensitivity of electron capture detection (ECD) and mass spectrometry (MS).

The first SRM with certified concentrations for individual PCB congeners was SRM 1588 (Organics in Cod Liver Oil). Measurement of the PCB congeners and pesticides in the cod liver oil required clean-up of the lipid matrix with a dimethylformamide/water/hexane liquid-liquid partitioning procedure followed by isolation of the PCBs and pesticides using a normal-phase LC procedure. The normal-phase LC procedure separates the analytes into two fractions, one containing the PCBs and lower polarity chlorinated pesticides (2,4'-DDE, 4,4'-DDE, and mirex) and the second fraction containing the more polar chlorinated pesticides. The separation of the PCBs and pesticides into two fractions

TABLE 12.18

CONCENTRATIONS OF PCB CONGENERS IN NATURAL MATRIX SRMs FOR THE MARINE ENVIRONMENT^a

Chloro- biphenyl number ^b	Name	Cod liver oil SRM 1588 (ng/g) ^c	River sediment SRM 1939 (ng/g) ^d	Marine sediment SRM 1941 (ng/g) ^e	Mussel tissue SRM 1974 (ng/g dry wt) ^f
PCB 18	(2,2',5'-Trichlorobiphenyl)			9.90 (0.25)	24 ± 9
15	(4,4'-Dichlorobiphenyl)				
PCB 26	(2,3',5'-Trichlorobiphenyl)		4200 ± 290		
PCB 28	(2,4,4'-Trichlorobiphenyl)	28.3 ± 0.6	2210 ± 100	16.1 (0.4)	62 ± 3
PCB 31	(2,4',5'-Trichlorobiphenyl)	8.3 ± 0.03	6860 (140)		53 (4)
PCB 44	(2,2',3,5'-Tetrachlorobiphenyl)	35.1 ± 1.4	1070 ± 120		5 ± 8
PCB 49	(2,2',4,5'-Tetrachlorobiphenyl)	29.8 ± 1.0			116 (1)
PCB 52	(2,2',5,5'-Tetrachlorobiphenyl)	83.3 ± 1.3	4480 (60)	10.4 (0.4)	98 ± 23
PCB 66	(2,3',4,4'-Tetrachlorobiphenyl)	54.8 ± 2.5 ^g	930 (10)	22.4 (0.7)	110 ± 5 ^g
95	(2,2',3,5',6-Pentachlorobiphenyl)	36.5 ± 0.8 ^g			17 (1)
PCB 99	(2,2',4,4',5-Pentachlorobiphenyl)	213 ± 2			45 (1)
PCB 101	(2,2',4,4',5,5'-Pentachlorobiphenyl)	129 ± 5	820 (10)	22.0 (0.7)	105 ± 11 ^h
90	(2,2',3,4',5-Pentachlorobiphenyl)				
PCB 105	(2,3,3',4,4'-Pentachlorobiphenyl)	60.2 ± 2.3		5.76 (0.23)	45 ± 3
PCB 110	(2,3,3',4',6-Pentachlorobiphenyl)	75.8 ± 1.9			94 (1)
PCB 118	(2,3',4,4',5-Pentachlorobiphenyl)	176 ± 2	510 (10)	15.2 (0.7)	110 ± 5
PCB 128	(2,2',3,3',4,4'-Hexachlorobiphenyl)	47.1 ± 2.6	100 (10)		15 ± 2
PCB 138	(2,2',3,4,4',5'-Hexachlorobiphenyl)	261 ± 29	570 (10)	24.9 (1.8)	110 ± 11
163	(2,3,3',4',5,6-Hexachlorobiphenyl)				
164	(2,3,3',4',5',6-Hexachlorobiphenyl)				

PCB 149	(2,2',3,4',5',6-Hexachlorobiphenyl)	105 ± 2			85 (1)
PCB 151	(2,2',3,5,5',6-Hexachlorobiphenyl)	55.2 ± 2.2			29 (1)
PCB 153	(2,2',4,4',5,5'-Hexachlorobiphenyl)	276 ± 40		22.0 (1.4)	145 ± 8
PCB 170	(2,2',3,3',4,4',5-Heptachlorobiphenyl)	45 ± 5	110 (10)	7.29 (0.26)	4.3 (0.1)
190	(2,3,3',4,4',5,6-Heptachlorobiphenyl)				
PCB 180	(2,2',3,4,4',5,5'-Heptachlorobiphenyl)	107 ± 4	160 (10)	14.3 (0.3)	13 ± 1
PCB 187	(2,2',3,4',5,5',6-Heptachlorobiphenyl)	35.3 ± 0.9	180 (10)	12.5 (0.6)	30 ± 1
182	(2,2',3,4,4',5,6'-Heptachlorobiphenyl)				
PCB 195	(2,2',3,3',4,4',5,6-Octachlorobiphenyl)			1.51 (0.10)	
208	(2,2',3,3',4,5,5',6,6'-Nonachlorobiphenyl)				
PCB 206	(2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl)			4.81 (0.15)	
PCB 209	(Decachlorobiphenyl)			8.35 (0.21)	

^aCertified concentrations in bold; see Certificate of Analysis for each SRM. Certified concentrations were obtained from the combined results of at least two independent analytical methods and the uncertainties are generally a 95% prediction interval with an allowance for systematic error among the methods used. Non-certified values are generally results from one analytical technique and the uncertainties (values in parentheses) are one standard deviation of a single measurement assuming all measurements are statistically independent and identically distributed.

^bPCBs are numbered according to Ballschmiter and Zell [53] except for PCB 201 which is numbered according to Schulte and Malisch [54]. Some PCB congeners are not separated in typical GC analyses; PCB congener listed first is the major component and additional congeners listed may be present as minor components.

^cSee ref. 28; non-certified concentrations for SRM 1588 are the mean of results from three techniques with uncertainties expressed as 95% confidence intervals.

^dSee ref. 29.

^eSee ref. 13.

^fSee ref. 14, non-certified values for SRM 1974 are the mean of results from three techniques with uncertainties expressed as 95% confidence intervals; values with uncertainties in parentheses are one standard deviation of a single measurement of one technique (see ref. 28).

^gPCB 95 and PCB 66 were separated in these analyses.

^hPCB 101 and PCB 90 were separated in these analyses.

TABLE 12.19

CONCENTRATIONS OF CHLORINATED PESTICIDES IN NATURAL MATRIX SRMs^a

Compound	Cod liver oil SRM 1588 (ng/g) ^b	River sediment SRM 1939 (ng/g) ^c	Marine sediment SRM 1941 (ng/g) ^d	Mussel tissue SRM 1974 (ng/g dry wt) ^e
Hexachlorobenzene	148 ± 21		2.0 (0.2)	
α-HCH	86 ± 19		5.8 (0.2)	
γ-HCH	25.5 ± 1.7		1.3 (0.1)	
trans-Chlordane	50 ± 13			
Heptachlor epoxide	33 ± 2	510 (20) ^f	0.23 (0.02)	26 (3)
cis-Chlordane	158 ± 8	220 (10)	2.06 (0.05)	26 ± 1
trans-Nonachlor	209 ± 11	270 (10)	0.97 (0.03)	21 ± 5
cis-Nonachlor	94.4 ± 1.4			
Dieldrin	150 ± 12		0.63 (0.03)	8 ± 4
2,4'-DDE	22.0 ± 1.1		5.8 ± 0.6	
4,4'-DDE	641 ± 62	540 (10)	9.71 (0.17)	48 ± 2
2,4'-DDD	36.3 ± 1.4		20 ± 7	
4,4'-DDD	277 ± 15	60 (10)	10.3 (0.1)	68 ± 3
2,4'-DDT	156 ± 5		4 ± 1	
4,4'-DDT	529 ± 45	1.11 (0.05)	3 ± 2	

^aCertified concentrations in bold; see Certificate of Analysis for each SRM. Certified concentrations were obtained from the combined results of at least two analytical methods and the uncertainties are generally a 95% prediction interval with an allowance for systematic error among the methods used. Non-certified values are generally results from one analytical technique and the uncertainties (values in parentheses) are one standard deviation of a single measurement assuming all measurements are statistically independent and identically distributed.

^bSee ref. 28; non-certified concentrations for SRM 1588 are the mean of results from three techniques with uncertainties expressed as 95% confidence intervals.

^cSee ref. 29.

^dSee ref. 13.

^eSee ref. 14; non-certified values for SRM 1974 are the mean of results from three techniques with uncertainties expressed as 95% confidence intervals; values with uncertainties in parentheses are one standard deviation of a single measurement of one technique (see ref. 28).

^fConcentration includes both heptachlor epoxide and oxychlordane.

reduces the possible co-elution of many of the pesticides with PCB congeners of interest. These two fractions were then analyzed by GC-ECD. The chromatograms from the GC-ECD analyses of these fractions from the cod liver oil SRM are illustrated in Figs. 12.5 and 12.6. For the second technique, GC-MS was used for quantitation after a size exclusion liquid chromatography procedure to remove most of the lipid material. These two techniques were used to provide certified concentrations for five PCB congeners and ten pesticides. These values have been confirmed recently using additional independent procedures, and additional analytes have been measured (see discussion below).

PCB congeners were certified in SRM 1939 using GC-ECD and a novel multidimensional GC-GC procedure with MS detection [29,55]. Because of the high levels of PCB congeners in SRM 1939, the extract could be analyzed directly by GC-ECD with no sample clean-up or isolation of the PCBs. In the multidimensional GC-GC procedure, a non-polar stationary phase (5% phenyl-substituted methylpolysiloxane), which is typically used for PCB separations, was used to provide separation of the PCB mixture. At selected times during this analysis, the effluent from this first column was directed onto a second column, a liquid crystalline stationary phase, to provide a very different selectivity for the separation of selected congeners that were not separated on the first column. Thus, the GC-GC procedure was used to resolve PCB congeners that co-elute in the procedures that are generally employed for PCB measurements. Only six PCB congeners were determined using the GC-GC procedure and 15 congeners were measured using the direct GC-ECD procedure. The results from these two techniques for the six congeners measured by both procedures are compared in Table 12.20. The results for three of the congeners (PCBs 26, 28 and 44) in Table 12.20 were in good agreement and certified values were determined. The results for PCB congeners 18 and 101 emphasize the need to have independent methods to determine the certified concentrations of PCB congeners. For PCB 18 the results from the GC-GC procedure are lower indicating the presence of co-eluting congeners (possibly PCB 15 and 17) when using the conventional GC-ECD procedure.

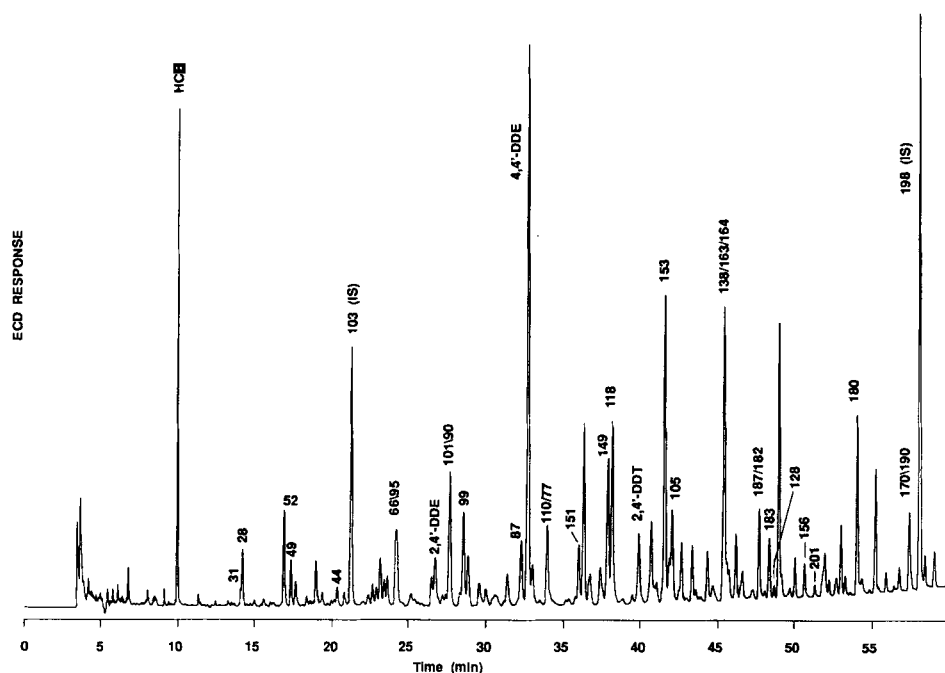


Fig. 12.5. Chromatogram from the GC-ECD analysis of the PCB and lower polarity pesticide fraction isolated from SRM 1588 (Organics in Cod Liver Oil). Column: 60-m capillary column with 5% phenyl-substituted methylpolysiloxane stationary phase (see ref. 27).

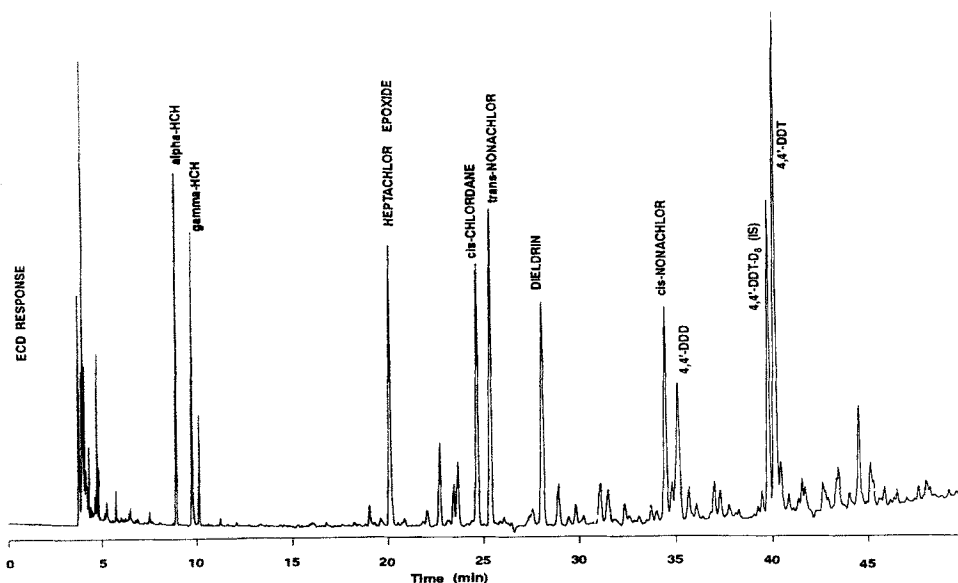


Fig. 12.6. Chromatogram from the GC-ECD analysis of the more polar chlorinated pesticide fraction isolated from SRM 1588 (Organics in Cod Liver Oil). Column: 60-m capillary column with 5% phenyl-substituted methylpolysiloxane stationary phase (see ref. 27).

Similarly for PCB 101, the GC-GC results were approximately one half of the results obtained with GC-ECD indicating the presence of a co-eluting congener, PCB 90, in this method [55].

SRMs 1941 and 1974 were originally issued with non-certified values for PCB congeners and pesticides based on measurements by GC-ECD only. Clean-up and isolation of the PCBs and pesticides were accomplished using the size exclusion LC (only for SRM 1974) and the normal-phase LC procedures described above for the cod liver oil. However, recently we have implemented the use of GC stationary phases with different selectivity and selective mass spectrometric detection for the measurement of PCB congeners and pesticides in environmental samples. This approach has been used to confirm the previous certified and non-certified concentrations for SRMs 1588, 1941 and 1974 and to provide results for additional analytes [28]. The results obtained using this approach for the measurement of PCB congeners in SRM 1588 are summarized in Table 12.21. The PCB fraction from SRM 1588 was analyzed using three GC stationary phases with different selectivity (5% phenyl-substituted methylpolysiloxane (DB-5), 5% phenyl-substituted methylpolysiloxane with 10% C-18 (C-18) and 14% cyanopropylphenyl-substituted methylpolysiloxane (DB-1701)) and two different GC detectors (electron capture (ECD) and mass spectrometric (MS)). As mentioned earlier, GC-ECD using a non-polar stationary phase such as 5% phenyl-substituted methylpolysiloxane is the typical method for the determination of PCB congeners; however, several of the PCB congeners of interest co-elute with other congeners on this column. The extent of the contribution of the co-eluting congeners has been shown to vary in different marine samples. Thus, the use of

TABLE 12.20

COMPARISON OF ANALYTICAL RESULTS ($\mu\text{g/g}$) FOR THE DETERMINATION OF PCB CONGENERS USING GC-ECD AND MULTIDIMENSIONAL GC-GC-MS

Congener	GC-ECD	GC-GC-MS	Certified
PCB 18	3.46 (0.08)	2.32 (0.06)	
17			
15			
PCB 26	4.24 (0.09)	4.16 (0.12)	4.20 ± 0.29
PCB 28	2.22 (0.04)	(2.20 (0.08)	2.21 ± 0.10
PCB 52	4.48 (0.06)	3.86 (0.05)	
PCB 44	1.09 (0.02)	1.04 (0.09)	1.07 ± 0.12
PCB 101	0.82 (0.01)	0.46 (0.03)	

^aUncertainties (values in parentheses) are one standard deviation of a single measurement.

additional stationary phases with differing selectivity for PCB separations is critical in the accurate determination of individual PCB congeners in environmental samples and particularly in establishing values on reference materials. Two of the columns used in this study, DB-5 and C-18, were non-polar stationary phases, but the C-18 column [56,57] exhibits separation behavior for the PCB congeners similar to that of the 50% *n*-octyl-methylpolysiloxane phase described previously [58,59]. The C-18 column separates the following congener pairs that normally co-elute on a 60-m 5% phenyl-substituted methylpolysiloxane column: PCB 15 from PCB 18, PCB 95 from PCB 66, PCB 90 from PCB 101, PCB 159 from PCB 187/182, and PCB 190 from PCB 170. The elution order of PCB 153 and PCB 105 is reversed on the C-18 column as compared to the 5% phenyl-substituted methylpolysiloxane. GC-MS analysis discriminates between co-eluting peaks with differences of an odd number of chlorine atoms (i.e. 1, 3, 5 and so on). Therefore, of the co-eluting congener pairs listed above, the GC-MS analysis can discriminate between PCB 15 and PCB 18, PCB 95 and PCB 66, and PCB 159 and PCB 182/187.

As shown in Table 12.21, the results obtained from the four different approaches are in good agreement, and discrepancies in the results can be attributed to the separation of co-eluting congeners due to differences in the selectivity of the columns or to the selectivity of the mass spectrometric detection. The results for several selected PCB congeners is discussed below to illustrate the necessity of such an approach for the accurate determination of PCB congeners in environmental reference materials.

PCB 66 (2,3',4,4'-tetrachlorobiphenyl) and PCB 95 (2,2',3,5',6-pentachlorobiphenyl), which are relatively major congeners in the cod liver oil sample with concentrations of each greater than 40 ng/g, co-elute on the DB-5 and DB-1701 columns. However, the C-18 column separates PCB 95 from PCB 66 and GC-MS selectively measures PCB 66 (tetrachloro substitution) in the presence of PCB 95 (pentachloro substitution), thereby providing measurements of these two individual congeners. PCB 101 (2,2',4,5,5'-pentachlorobiphenyl) and PCB 90 (2,2',3,4',5-pentachlorobiphenyl) are separated on the C-18

TABLE 12.21

CONCENTRATIONS (NG/G) OF SELECTED PCB CONGENERS IN SRM 1588, ORGANICS IN COD LIVER OIL, AS DETERMINED AT NIST BY USING GC-ECD AND GC-MS

Polychlorinated biphenyls ^a		Certified ^b	GC-ECD (DB-5) ^c	GC-ECD (C-18) ^d	GC-ECD (DB-1701) ^e	GC-MS (DB-5) ^f	Recommended value ^g
PCB 28	(2,4,4'-Trichlorobiphenyl)		28.1 (0.4)	29.2 (1.2)	27.4 (1.3)	28.0 (1.4)	28.3 ± 0.6
PCB 31	(2,4',5-Trichlorobiphenyl)		8.1 (0.4)	8.4 (0.9)	8.9 (1.4)	8.2 (1.2)	8.3 ± 0.3
PCB 44	(2,2',3,5'-Tetrachlorobiphenyl)		34.2 (1.4)	37.0 (1.2)	32.3 (1.3)	35.4 (1.9)	35.1 ± 1.4
PCB 49	(2,2',4,5'-Tetrachlorobiphenyl)		29.3 (0.7)	29.9 (1.4)	32.2 (0.9)	29.0 (1.2)	29.8 ± 1.0
PCB 52	(2,2',5,5'-Tetrachlorobiphenyl)		81.1 (3.5)	82.6 (5.6)	85.0 (2.5)	85.4 (2.9)	83.3 ± 1.3
PCB 66	(2,3',4,4'-Tetrachlorobiphenyl)		[95.4 (2.7)] ^h	53.4 (1.8)	[100.2 (3.7)] ^h	56.1 (1.6)	54.8 ± 2.5
PCB 87	(2,2',3,4,5'-Pentachlorobiphenyl)		55.6 (2.2)	56.4 (2.2)	56.7 (1.8)	56.8 (1.2)	56.3 ± 0.6
PCB 95	(2,2',3,5',6-Pentachlorobiphenyl)		[95.4 (2.7)] ^h	37.1 (2.2)	[100.2 (3.7)] ^h	35.9 (1.3)	36.5 ± 0.8
PCB 99	(2,2',4,4',5-Pentachlorobiphenyl)		213 (5)	214 (5)	209 (5)	213 (6)	213 ± 2
PCB 101	(2,2',4,5,5'-Pentachlorobiphenyl)	129 ± 5	[126 (7)] ⁱ	124 (7)	125 (3)	[131 (4)] ⁱ	127 ± 2
PCB 105	(2,3,3',4,4'-Pentachlorobiphenyl)		63.2 (6.0)	56.7 (4.6)	61.0 (2.4)	60.4 (2.5)	60.2 ± 2.3
PCB 110	(2,3,3',4',6-Pentachlorobiphenyl)		[89.5 (4.2)] ^j	76.4 (1.9)	73.3 (3.1)	76.4 (2.0)	75.8 ± 1.9
PCB 118	(2,3',4,4',5-Pentachlorobiphenyl)		179 (8)	175 (9)	177 (5)	174 (4)	176 ± 2
PCB 128	(2,2',3,3',4,4'-Hexachlorobiphenyl)		48.0 (1.3)	47.9 (1.4)	40.4 (1.6)	49.0 (2.5)	47.1 ± 2.6
PCB 138	(2,2',3,4,4',5'-Hexachlorobiphenyl)	261 ± 29	265 (16)	258 (10)	274 (8)	261 (20)	263 ± 5
163	(2,3,3',4',5,6-Hexachlorobiphenyl)						
164	(2,3,3',4',5',6-Hexachlorobiphenyl)						
PCB 149	(2,2',3,4',5',6-Hexachlorobiphenyl)		105 (3)	103 (3)	110 (5)	105 (5)	105 ± 2
PCB 151	(2,2',3,5,5',6-Hexachlorobiphenyl)		52.4 (3.3)	57.0 (2.5)	59.2 (1.7)	54.4 (3.4)	55.2 ± 2.2
PCB 153	(2,2',4,4',5,5'-Hexachlorobiphenyl)	276 ± 40	281 (15)	278 (11)	264 (9)	277 (11)	277 ± 5

PCB 156	(2,3,3',4,4',5-Hexachlorobiphenyl)		27.5 (6.9)	29.3 (8.7)	23.4 (0.8)	27.2 (4.8)	27.3 ± 2.0
PCB 170	(2,2',3,3',4,4',5-Heptachlorobiphenyl)	45 ± 5	48.3 (3.5)	45.8 (2.8)	44.4 (1.8)	46.3 (1.8)	46.4 ± 1.2
190	(2,3,3',4,4',5,6-Heptachlorobiphenyl)						
PCB 180	(2,2',3,4,4',5,5'-Heptachlorobiphenyl)	107 ± 4	107 (4)	104 (4)	107 (3)	107 (2)	106 ± 1
PCB 183	(2,2',3,4,4',5',6-Heptachlorobiphenyl)		30.4 (2.0)	31.4 (1.3)	32.1 (2.0)	30.0 (1.3)	30.8 ± 0.7
PCB 187	(2,2',3,4,4',5,5',6-Heptachlorobiphenyl)		36.1 (1.7)	34.7 (2.2)	37.1 (1.5)	34.3 (1.7)	35.3 ± 0.9
182	(2,2',3,4,4',5,6'-Heptachlorobiphenyl)						
PCB 194	(2,2',3,3',4,4',5,5'-Octachlorobiphenyl)		15.4 (1.2)	16.2 (1.2)	13.8 (1.0)	16.4 (1.1)	15.7 ± 0.8
PCB 201	(2,2',3,3',4,5',6,6'-Octachlorobiphenyl)		12.4 (1.3)	12.4 (1.2)	13.0 (1.2)	11.4 (1.2)	12.2 ± 0.5

^aPCBs are numbered according to Ballschmiter and Zell [53] except for PCB 201 which is numbered according to Schulte and Malisch [54]; PCB congener listed first is the major component; additional PCB congeners listed may be present as minor components.

^bCertified concentrations with uncertainties expressed as two standard deviations; see Certificate of Analysis.

^cConcentrations determined using GC-ECD on a DB-5 column. Twelve aliquots of SRM 1588 analyzed in duplicate; concentration value is the mean value, and the numbers in parentheses are one standard deviation of a single measurement.

^dConcentrations determined using GC-ECD on a C-18 column. Twelve aliquots of SRM 1588 analyzed in duplicate; concentration value is the mean value, and the numbers in parentheses are one standard deviation of a single measurement.

^eConcentrations determined using GC-ECD on a DB-1701 column. Six aliquots of SRM 1588 analyzed in duplicate; concentration value is the mean value, and the numbers in parentheses are one standard deviation of a single measurement.

^fConcentrations determined using GC-MS on a DB-5 column. Twelve aliquots of SRM 1588 analyzed in duplicate; concentration value is the mean value, and the numbers in parentheses are one standard deviation of a single measurement.

^gRecommended values are mean values from analysis of the same samples using four methods with the uncertainties expressed as 95% confidence intervals. Where certified values exist for a congener, the recommended value is not intended to replace the certified value but only represents recent measurements using the four different techniques.

^hValues in [] indicate known co-elution of two or more congeners; PCB 95 co-eluted with PCB 66.

ⁱValues in [] indicate known co-elution of two or more congeners; PCB 90 co-eluted with PCB 101.

^jValues in [] indicate known co-elution of two or more congeners; PCB 77 (3,3',4,4'-tetrachlorobiphenyl) co-eluted with PCB 110.

column and on the DB-1701 column but are not separated on the DB-5 column and are not distinguishable by GC-MS. SRM 1588 has very little PCB 90 as indicated by the concentration of 124 ng/g for PCB 101 determined using the C-18 column compared to the certified concentration of 129 ng/g for the combined PCB 101/90. In SRM 1974, the concentration of PCB 90 is 25% of the concentration of PCB 101 [28]. PCB 110 (2,3,3',4',6-pentachlorobiphenyl) co-eluted with the "planar" congener PCB 77 (3,3',4,4'-tetrachlorobiphenyl) when using the DB-5 column. The "planar non-ortho"-substituted PCB congeners are of particular interest due to their enhanced toxicity [56]. The "planar" PCBs were not quantified in this study, but work is ongoing in our laboratory to isolate and quantify these compounds in these reference materials. PCB 110 and PCB 77 are separated using the C-18 and DB-1701 column and are distinguishable by GC-MS. In SRM 1588, the concentration of PCB 77 is approximately 15% of the concentration of PCB 110. PCB 170 (2,2',3,3',4,4',5-heptachlorobiphenyl) and PCB 190 (2,3,3',4,4',5,6-heptachlorobiphenyl) are not separated on the DB-5 or DB-1701 column and are not distinguishable by GC-MS. However, this congener pair is separated on the C-18 column. In SRM 1588, the concentration of PCB 190 is less than 5% that of PCB 170. However, in other fish oil reference materials (i.e. CRM 349 and 350 from the Community Bureau of Reference), the concentration of PCB 190 is 10–25% that of PCB 170 [28]. PCB 187 (2,2',3,4',5,5',6-heptachlorobiphenyl), PCB 182 (2,2',3,4,4',5,6'-heptachlorobiphenyl) and PCB 159 (2,3,3',4,5,5'-hexachlorobiphenyl) co-elute on the DB-5 column. The C-18 column and the DB-1701 column separates PCB 159 from PCB 187/182 while the GC-MS selectively measures PCB 159 (hexachloro substitution) in the presence of PCB 187/182 (heptachloro substitution). In SRM 1588 and SRM 1974, the contribution of PCB 159 is negligible. However, in SRM 1941 and in CRM 349 and CRM 350, the concentration of PCB 159 is approximately 5–10% that of PCB 187/182.

As illustrated by the above examples, the combination of different stationary phases and selective mass spectrometric detection is necessary to achieve reliable measurements of PCB congeners in environmental matrices. This approach has been used to re-analyze SRMs 1588, 1941 and 1974, and CRMs 349 and 350 and the results are reported by Schantz et al. [28]. This approach using different columns with different selectivity and GC-MS will be the basis for future certifications of PCB congeners and chlorinated pesticides in environmental matrix SRMs.

12.2.4. SRM activities in progress

12.2.4.1. *Re-issue of sediment and tissue SRMs*

Since the marine sediment and mussel tissue SRMs were issued in 1989 and 1990, they have found widespread use in marine monitoring programs. As a result, the supplies of SRMs 1941 and 1974 are anticipated to be depleted in 1993 and 1994, respectively. A new sediment material was collected in 1991 at the same Baltimore Harbor location and has been processed as a replacement for SRM 1941. This new sediment material is currently being analyzed to provide certified and non-certified concentrations for aliphatic hydrocarbons, PAHs, PCBs and chlorinated pesticides and will be issued early in 1993 as SRM 1941a. A new mussel tissue material has been collected and will be processed and

analyzed in 1993 to provide a replacement for SRM 1974. Mussels were collected from the same site in Boston Harbor to provide a material similar to SRM 1974. The proposed list of certified analytes for this new mussel material will be expanded beyond PAHs to also include aliphatic hydrocarbons, PCBs and chlorinated pesticides.

12.2.4.2. Whale blubber SRM

Because of increasing concern about the effects of pollution on the health of marine mammals, many laboratories are involved in the analysis of marine mammal tissues to measure the levels of organic and inorganic contaminants. As a result, NIST is preparing a whale blubber SRM for use in validating measurements of PCB congeners and chlorinated pesticides. A pilot batch of whale blubber was prepared and analyzed to determine the feasibility of preparing the whale blubber SRM [60]. In September 1991, 15 kg of blubber was collected from a mass stranding of pilot whales (*Globicephala melaena*) on Cape Cod, MA. The blubber material was cryogenically pulverized and homogenized to produce a frozen blubber homogenate which will be issued in late 1992 as SRM 1945 (Organics in Whale Blubber). This SRM will have certified concentrations of 20–30 PCB congeners and chlorinated pesticides with concentrations of approximately 50–400 ng/g wet weight for the major analytes.

12.2.4.3. Recertification of air and diesel particulate SRMs

SRM 1649, issued in 1982, was one of the first natural matrix environmental SRMs developed at NIST for the measurement of organic constituents. This SRM has certified concentrations for only five PAHs and non-certified values for nine additional PAHs. SRM 1650 was issued in 1985 and has certified and non-certified concentrations for a similar number of PAHs and nitro-PAHs. Since these two materials were issued, we have implemented improved and/or additional methods for the measurement of a large number of PAHs. Using current methodologies these two SRMs will be re-analyzed to provide certified concentrations for 15–20 PAHs and non-certified values for an additional 15–20 PAHs. By significantly increasing the number of certified and non-certified concentrations for PAHs in SRM 1649 and SRM 1650, these materials will be more useful to the organic analytical community.

12.2.4.4. Diesel particulate extract SRM

To eliminate the variability associated with extraction of particulate samples, the analytical environmental chemistry and the bioassay communities have expressed interest in the development of an SRM that is an extract of diesel particulate matter or air particulate matter to complement the existing natural matrix SRMs that require extraction. Bulk quantities of a new diesel particulate material with relatively high mutagenic activity have been obtained for this SRM. SRM 1975 (Diesel Particulate Extract) will be characterized for selected PAHs and nitro-PAHs as well as for mutagenic activity.

12.3. USES OF ENVIRONMENTAL SRMs

The SRMs described above are intended for use in analytical methods development and validation and to assure long-term measurement comparability. The natural matrix materials are ideally suited for use in developing and validating new methods. These SRMs can be analyzed on a regular basis in conjunction with unknown samples with similar matrices to continually monitor the accuracy and precision of the analytical procedures.

Natural matrix SRMs have also been found to be useful for methods development for the measurement of compounds that have not been certified or measured at NIST. In this instance, SRMs are homogeneous natural environmental matrices that are readily available to other laboratories for comparison of analytical results. As laboratories involved in environmental pollution monitoring expand the number of PAHs or PCB congeners measured due to emphasis on toxicity, pollution source identification, etc., published results of analyses of these SRMs by other laboratories will provide a database for comparison within the scientific community. For example, even though these materials have not been analyzed at NIST for PCDDs, PCDFs or planar PCB congeners, they offer convenient matrices for use in addressing these analytes.

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Chapter 13

Application of fluorescence spectroscopic techniques in the determination of PAHs and PAH metabolites

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13.1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are usually defined as a group of chemicals consisting of two or more fused benzenoid rings and containing no other elements than hydrogen and carbon. In general, they are highly fluorescent, and fluorescence spectroscopic techniques are frequently used for the analysis of these compounds.

PAHs are natural constituents of crude oil and many other petrochemical products. These aromatic structures have been formed during the course of millions of years by successive dehydrogenation of organic material at relatively low temperatures. In PAH mixtures of petrochemical origin, two- or three-ring compounds, such as naphthalene, phenanthrene and their alkylated derivatives, are more abundant than the heavier PAHs containing four or more rings.

PAHs can also be formed at more elevated temperatures during the inefficient combustion of fossil fuels or other organic matter. A typical PAH mixture of a high-temperature combustion source contains mainly unsubstituted compounds, and the PAHs of four and more rings are more abundant than the smaller ones. PAHs in natural samples are always encountered as mixtures; the relative distribution of the various PAH congeners, the PAH "profile", provides a clue to the origin (petrochemical or pyrolytic) of the pollution.

Natural sources of PAH emission have always existed (sediment erosion, volcanic eruptions, forest fires), but since the industrial revolution there has been a rapid increase in the loading of the environment with petrochemical PAHs (oil spills) and particularly with PAHs of pyrolytic origin.

Many PAHs are suspected carcinogens, the most well-known example being the five-ring aromatic compound benzo[*a*]pyrene (BaP). It should be realized, however, that BaP itself is relatively inert; it needs to be metabolized before it can exert genotoxic activity. Biotransformation starts with the binding of the xenobiotic compounds to the cytochrome *P*-450 enzyme system, which catalyzes the addition of an oxygen atom across a double bond of the molecule, thus forming an epoxide. This epoxide may subsequently be coupled to glutathione, isomerize into a phenol or be hydrolyzed to yield a saturated dihydrodiol moiety. Phenols and dihydrodiols can be conjugated to glucuronic acid or sulfate to facilitate excretion. Overall, the biotransformation of the strongly lipophilic BaP results in the formation of more polar metabolites that are more easily excreted than the parent compound. The result of these detoxification mechanisms is that the bulk of the PAH molecules, after absorption by higher species, are also rapidly removed from the body. However, some reactive intermediates formed during the process may form stable adducts with proteins or with DNA. The latter could lead to the initiation of cancer if the defective nucleotide is not repaired in time.

The challenge for analytical chemists is not only to develop methods for the identification and quantitation of PAHs in various environmental samples; the amount of a specific PAH that is actually absorbed by a given organism also needs to be determined. In this chapter, the attention is focussed on the development and application of fluorescence techniques for this purpose. The two spectroscopic methods described can be applied to whole samples without the need for chromatographic separation. Since conventional fluorescence spectroscopy is not appropriate for the analysis of mixtures of PAHs in real samples, enhancement of selectivity has to be realized. Two approaches are followed, i.e. Shpol'skii spectroscopy, a technique providing highly specific fluorescence spectra (with fingerprinting characteristics) for certain analytes in (poly)crystalline matrices at cryogenic temperatures, and synchronous fluorescence spectroscopy (SFS), a conventional, room temperature technique based on the synchronous scanning of excitation and emission wavelengths.

Extensive attention is given to applications of Shpol'skii spectroscopy and SFS to marine environmental analysis. The Shpol'skii method is applied to the determination of parent PAHs in sediment and biota samples. Even more important is its application to the identification and quantitation of BaP metabolites in bile of fish exposed to sediments with different PAH pollution levels. Thus, a direct indication of the amount of BaP entering the body can be obtained. SFS is not sensitive enough to detect BaP metabolites in fish bile. Nevertheless, a correlation was found between 3-hydroxy-BaP and 1-hydroxy-

pyrene concentrations, and the latter can be readily determined with SFS. Although pyrene is not believed to be particularly toxic, the determination of its major metabolite 1-hydroxy pyrene in bile samples by means of SFS would be a suitable screening method for the biomonitoring of total PAH exposure, provided the PAH uptake profile is more or less constant.

13.2. FLUORESCENCE SPECTROSCOPY

When solid or fluid solutions are irradiated with ultraviolet or visible light, sometimes luminescence (fluorescence and/or phosphorescence) is observed, i.e. the sample emits light of longer wavelength than the excitation light in all directions. For a solute molecule, the energy diagram, usually denoted the Jablonski diagram, and the relevant transitions and decay processes are depicted in Fig. 13.1. Through the absorption of light, molecules (or atoms) may be promoted to a higher electronic state (S_1 , S_2 , etc.). The energies and probabilities of these transitions can be studied by recording an (electronic) absorption spectrum. In the condensed phase, relaxation of the excited molecule to the lowest vibrational level of the S_1 state (via internal conversion and vibrational relaxation), is usually very fast. The remaining excitation energy stored in the molecule may also be lost non-radiatively, or may be transferred intermolecularly to other chromophoric groups in the sample or intramolecularly within the same molecule. For a limited number of compounds, however, the return from S_1 to the electronic ground state S_0 is accompanied by the emission of a photon (fluorescence). In some cases, phosphorescence from the lowest vibrational level of the triplet state T_1 takes place. Fluorescence and phosphorescence

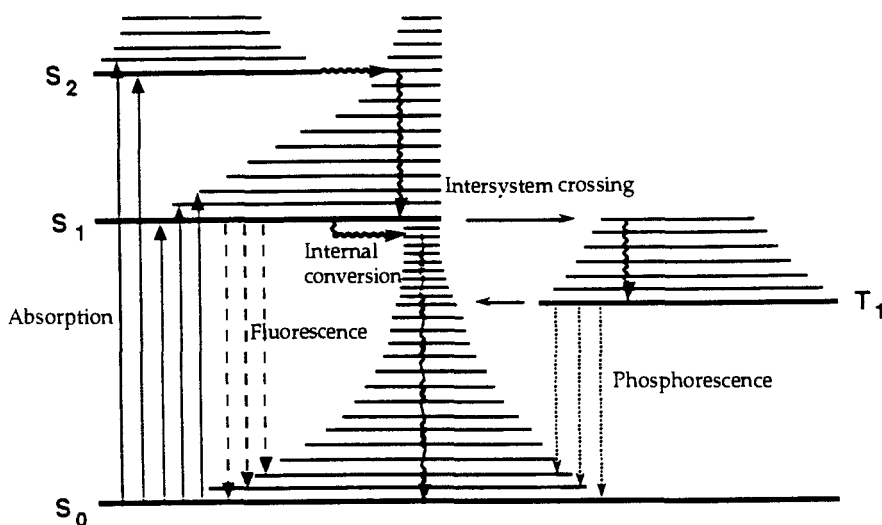


Fig. 13.1. Jablonski diagram of molecular system, showing relevant electronic transitions and decay processes. S, singlet state; T, triplet state. Reproduced from [2] with permission.

spectra yield information on the vibrational levels of the electronic ground state S_0 , and are as such complementary to the absorption spectrum.

Luminescence spectroscopic techniques are widely recognized as useful tools in analytical chemistry, especially because of their high inherent sensitivity (zero-background measurement). Obviously, the fact that only a restricted group of analytes displays fluorescence implies that the method is on the one hand not universally applicable, but at the same time more selective. Polycyclic aromatic hydrocarbons usually show strong fluorescence, and several fluorescence-based techniques have been developed to study PAHs for analytical purposes. For the determination of mixtures of PAHs, for instance in petrochemical or environmental samples, fluorimetric detection usually takes place after some form of (chromatographic) separation. Separation is necessary, as (rather unexpected in view of the discrete levels shown in Fig. 13.1) the fluorescence spectra of molecules often consist of rather broad bands that provide little information on the identity of the compound. Furthermore, fluorescence spectra of different compounds often show severe overlap. Identification and proper quantitation of analytes in a mixture is not possible under conventional conditions.

A simple method that leads to some spectral simplification and reduced overlap is known as synchronous fluorescence spectrometry (SFS). A more significant increase in spectral resolution may be obtained by means of several low-temperature techniques: fluorescence line-narrowing spectroscopy; supersonic jet spectroscopy; matrix isolation spectroscopy; or Shpol'skii spectroscopy (SS). For the analysis of PAHs and PAH metabolites, the Shpol'skii technique proved particularly useful, as demonstrated below.

13.2.1. Shpol'skii spectroscopy

In order to understand the Shpol'skii effect, the question should be answered why, for molecules in the condensed liquid or solid phase, broad-banded spectra are observed instead of discrete, sharp lines. Irrespective of other mechanisms, the main cause is inhomogeneous line broadening: each individual analyte molecule experiences a different influence of its surrounding solvent cage. Thus, each molecule has its own Jablonski diagram and the energies of the electronic states are slightly different. As a result, a Gaussian distribution of narrow lines is observed with a total band width of typically several hundred cm^{-1} for each transition.

In Shpol'skii spectroscopy, the inhomogeneous broadening induced by the matrix is largely diminished. Shpol'skii and co-workers [1] observed a dramatic line-narrowing effect in the fluorescence spectrum of coronene on rapid cooling to 77 K in *n*-hexane or *n*-heptane solutions. The use of organic solvents that form amorphous, glassy matrices at low temperatures did not produce high-resolution spectra. The phenomenon, which soon became known as the Shpol'skii effect, is illustrated in Fig. 13.2a and b, which depicts the fluorescence spectra of benzo[*k*]fluoranthene in *n*-octane at room temperature and at 26 K, using the same experimental setup [2]. Since the total fluorescence intensity is at least equal and often even higher at low temperatures, the line-narrowing effect results at the same time in an increase in signal height of two orders of magnitude. The sensitivity of the Shpol'skii technique is discussed in more detail in Section 13.3.

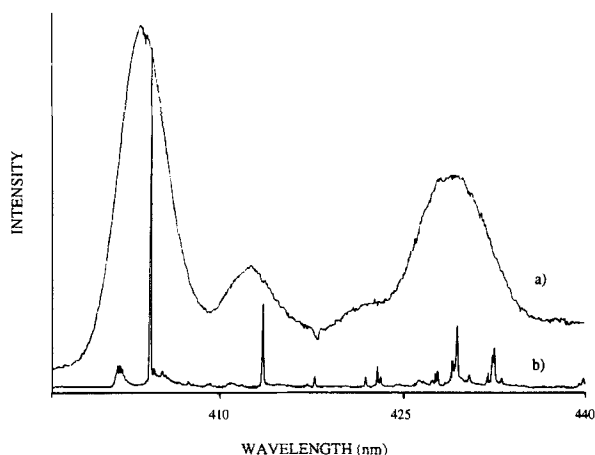


Fig. 13.2. Emission spectra of benzo[*k*]fluoranthene in *n*-octane, using xenon lamp excitation at 308 nm. (a) Room temperature spectrum, concentration = 10^{-4} M. (b) Shpol'skii spectrum at 26 K, concentration = 10^{-6} M. Spectra were recorded using the same experimental settings; intensities are on the same scale. Reproduced from [2] with permission.

It is generally believed that the narrow-banded or quasilinear emission lines are produced by isolated molecules, trapped in the matrix during the cooling procedure and substituting one or a few solvent molecules in the (poly)crystalline lattice [3–5]. When the analyte can occupy two or more different sites in the matrix, a multiplet spectrum will be observed, as the individual spectra of different subsets of molecules are shifted with respect to each other as the result of different matrix interactions (different 0–0 energies). The Shpol'skii spectrum of 1-hydroxy-benzo[*a*]pyrene in *n*-octane (Fig. 13.3) is an example of a doublet spectrum [2]. The intensity ratio of the various emission lines within a multiplet is the same for each transition and reflects the distribution of the analyte molecules over the various sites. When a narrow-banded excitation source (e.g. a dye laser) is tuned to a narrow-banded absorption transition of one of the analyte subsets, a single-site spectrum will be observed, as was first demonstrated by Vo-Dinh and Wild [6].

The spectral band widths that can be observed in Shpol'skii systems at temperatures of 20 K and lower are typically $2\text{--}10\text{ cm}^{-1}$ (ca. 0.1 nm). Abram and co-workers [7] showed that the line width of a vibronic emission band of perylene in *n*-octane at 4.2 K could be reduced from 4 cm^{-1} to the instrumental limit of 0.4 cm^{-1} by selective excitation with a laser. This illustrates that some inhomogeneous broadening is still present in Shpol'skii systems. In practice, however, the line-narrowing induced by the matrix alone (in a lamp excited experiment) is often sufficient for isomer-specific identification and for the determination of complex mixtures.

Whether a given analyte will produce Shpol'skii-like emission in a particular matrix depends on the compatibility of the physical and geometrical properties of the host-guest combination. For instance, naphthalene “fits” in an *n*-pentane crystal, but yields only broad-banded emission in *n*-hexane and *n*-heptane. This phenomenon is usually referred to as the “key and hole principle”, although it appears that the geometric requirements are

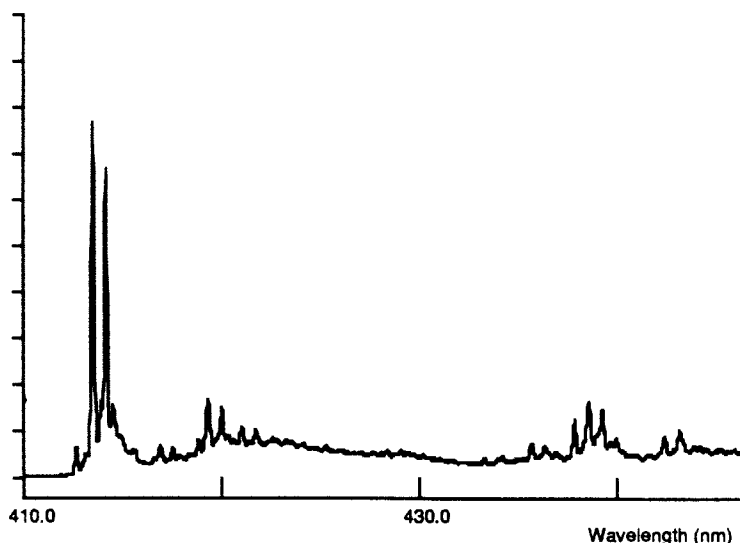


Fig. 13.3. Double-site spectrum of 1-hydroxy-benzo[a]pyrene in *n*-octane at 10 K; concentration 5×10^{-6} M; xenon lamp excitation 295 nm. Reproduced from [2] with permission.

less stringent for most larger PAHs containing four or more rings, as they produce good Shpol'skii spectra in a range of *n*-alkane matrices [8,4].

Schematically, an instrumental setup for Shpol'skii fluorescence measurements consists of the following modular components: (1) excitation source, often with some type of wavelength selector; (2) low temperature sample holder; (3) high-resolution emission monochromator; (4) detector. At present, no complete turn-key instruments are available on the market, but a Shpol'skii setup can be assembled from commercially available components.

13.2.1.1. Excitation sources

The Shpol'skii effect is a matrix-induced phenomenon; the use of highly monochromatic (laser) excitation can be advantageous, but is not a prerequisite for observing narrow-banded spectra. Frequently mercury or xenon arc lamps in combination with an excitation monochromator are applied. Laser sources are utilized to improve selectivity and/or sensitivity. For PAHs it is known that also the S_1-S_0 part of the absorption spectrum consists of narrow lines. Selective excitation is possible if the laser wavelength can be tuned to a specific absorption line of the particular analyte under investigation. Nowadays, several types of lasers and dye lasers are available which – in combination with frequency doubling, frequency tripling, frequency sum mixing or Raman shifting techniques – cover a wide range of relevant wavelengths. For the laser excited Shpol'skii experiments described below [2], an Nd:YAG laser (frequency doubled) was used in combination with a dye laser. For the measurements of BaP metabolites the Nd:YAG setup could be easily switched from frequency mixing of the dye laser output (for selective excitation around 420 nm) to frequency doubling (for non-selective excitation around 350 nm).

13.2.1.2. Cryostats

Although the Shpol'skii effect was first observed at a temperature of 77 K, the boiling point of liquid nitrogen, the use of lower temperatures results in a substantially better spectral resolution [2]. Various types of helium cryostats are available; the closed-cycle systems have become very popular because of their ease and low cost of operation (no helium consumption). In these cryostats, the circulating medium helium is expanded in the cold station and cools the sample through thermal conductance. Depending on the number of cold stages of the instrument, temperatures of typically 10 or 20 K can be reached. The cooling procedure of the sample obviously affects the freezing rate of the sample, and may thus have an influence on the shape of the spectrum for certain analytes that are not fully compatible with the matrix. Which method results in the most efficient trapping of isolated molecules in crystalline sites will also depend on the sample holder design [9].

13.2.1.3. Emission monochromators

Evidently, a good monochromator of moderate or high resolution is required to observe the quasilinear emission spectrum. The spectral resolution of the instrument should preferably be better than 0.2 nm. For trace analysis, the instrument should also have a high light throughput. Holographic gratings, optimized for the wavelength area of interest, are preferred. Often the F/n number of the emission monochromator will be fairly high, which means that some care should be taken with proper focussing of the fluorescence light on the entrance slit.

If non-selective, short-wavelength excitation is applied, scattered excitation light is easily rejected with an appropriate cut-off filter. If, on the other hand, the excitation wavelength is close to the emission lines of interest, as in the case of selective laser excitation in the S_1 - S_0 absorption region, the cut-off functions of such filters are usually not sufficiently steep. In that case, the use of a double or even triple monochromator will be very advantageous. For the laser excited Shpol'skii spectroscopy (LESS) measurements described below [2], a Spex 1877 triple monochromator was used.

13.2.1.4. Detectors

Until about 5 years ago, photomultiplier tubes (PMTs) in combination with a scanning monochromator were usually used for detection. However, they have the obvious disadvantage that the time required to record a complete high-resolution spectrum is long (typically 10–30 min).

More recently, multichannel detectors have become available that are particularly useful in high-resolution spectroscopy. These detectors are mounted in the (exit) focal plane of the monochromator after removal of the exit slit. The monochromator is thus turned into a spectrograph; measurements are usually carried out at a fixed wavelength position, although operating the detector in the scanning mode can have certain advantages [10]. The intensified linear diode array (ILDA) detector consists of typically 512 or 1024 separate photodiodes (center to center distance ca. 25 μm), that release electrons on the ab-

sorption of light. At the end of an integration cycle defined by the operator, the accumulated charge in each photodiode-capacitor pair is sampled, digitized and fed into a computer. Since the read-out of such detectors is accompanied by considerable electronic noise, the use of an intensifier (a combination of a photocathode, a multichannel plate and a phosphor screen, which multiplies the number of incident photons with a gain of typically 10^3) is indispensable for the detection of low light levels. Fast gating of the ILDA detector is possible by means of a fast pulser unit that switches the photocathode of the intensifier unit on and off at specific times.

Another type of multichannel detector is the charge-coupled device (CCD) detector. These detector chips consist of a plane of semiconductor material and a fine electronic network that divides the chip into a matrix of small squares (pixels). Typical CCD chips presently contain 512×512 pixels of approximately $20 \times 20 \mu\text{m}$ each. As the read-out noise of CCD chips is rather low, CCD detectors do not necessarily require an intensifier. With CCD detectors two-dimensional spectra can be recorded [11], but in the case of ordinary one-dimensional spectroscopic measurements, the charge accumulated in each column can be summed ("binned") for extra sensitivity. A scintillator dye can be applied to the CCD chip for extension into the UV region.

In our experience ILDA and CCD detectors offer roughly comparable sensitivities, when applied to Shpol'skii analysis [2]. In the experiments described below, an ILDA was used. When compared to PMT detection, it should be noted that not only the gain in analysis time (ca. 2 orders of magnitude) offered by the multichannel detector plays a role; an even more important advantage of the multichannel detector is that each data point of the complete spectrum is equally affected by light source instability (slow drift, flicker noise) or by photochemical decomposition of the analyte. These factors will be particularly important when lasers are used for excitation.

13.2.2. Synchronous fluorescence spectroscopy

In conventional molecular fluorescence spectroscopy, two types of spectra are generally discerned: emission and excitation spectra. In order to record an emission spectrum, the compound is excited at a fixed wavelength, while the fluorescence intensity is measured as a function of the emission wavelength. At ambient or lower temperatures, fluorescence emission of virtually all PAHs takes place after relaxation of the excited molecule to the lowest vibrational state of the first excited singlet state S_1 (see the Jablonski diagram, Fig. 13.1). Thus, the shape of the emission spectrum is independent of the excitation wavelength and only reflects the S_1 - S_0 energy difference, the vibrational levels of the S_0 ground state, and the respective transition probabilities. The excitation wavelength thus affects only the absolute intensity of the emission spectrum.

Alternatively, an excitation spectrum can be recorded by measuring the fluorescence intensity at a fixed wavelength, while scanning the wavelength of the excitation light. The absolute intensity of the excitation spectrum may be influenced by the choice of the wavelength monitored, but the shape of the excitation spectrum is emission-independent and only reflects the transitions and transition probabilities from the lowest vibrational level of the S_0 electronic ground state to vibrational levels of excited singlet states (S_1 and higher).

If the excitation wavelength λ_{ex} and the emission wavelength λ_{em} are both varied, the total luminescence function is obtained, which is a multiplication of E_{ex} (a function describing the emission spectrum) and E_{em} (a function describing the excitation spectrum). The total luminescence constitutes a three-dimensional hypersurface, that can be visualized as a pseudo-three-dimensional stack plot or, alternatively, as a contour plot projected onto the $\lambda_{\text{ex}}-\lambda_{\text{em}}$ plane, in which contour lines connect points of equal intensity I (Fig. 13.4). A vertical cross-section through the TL plot produces the conventional excitation spectrum ($\lambda_{\text{em}} = \text{constant}$), while a horizontal cross-section parallel to the λ_{em} axis yields the conventional emission spectrum.

We now use the total luminescence plot of Fig. 13.4 to visualize the most important advantages of the synchronous scanning technique. One can easily imagine that different cross-sections through the TL surface can be obtained if λ_{ex} and λ_{em} are both allowed to vary during the experiment. Although modern spectrofluorimeters with software-driven, independent monochromators could offer an unlimited number of scanning combinations, the traditional approach, as described first by Lloyd [12,13], involved a spectrofluorimeter in which the excitation and emission monochromators were mechanically interlocked, such that $\lambda_{\text{ex}} - \lambda_{\text{em}} = \text{constant}$. The fluorescence intensity is recorded as the excitation wavelength trails the plotted emission. Lloyd called the spectra obtained in this way "synchronously excited fluorescence emission spectra", although they could also be regarded as excitation spectra with synchronously recorded emission [14]. In Fig. 13.4, the SFS spectrum is represented by a diagonal cross-section through the TL surface, along the line $\lambda_{\text{em}} - \lambda_{\text{ex}} = \Delta\lambda = \text{constant}$:

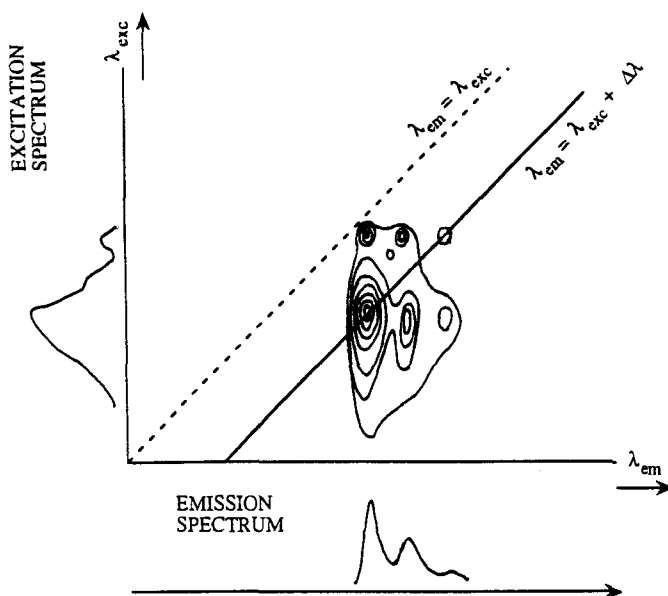


Fig. 13.4. Contour plot of total excitation-emission function of compound showing typical PAH vibrational structure. The synchronous spectrum is represented by the diagonal cross-section $\lambda_{\text{em}} = \lambda_{\text{ex}} + \Delta\lambda$. Rayleigh scattering occurs at $\lambda_{\text{em}} = \lambda_{\text{ex}}$. Reproduced from [2] with permission.

$$I_{\text{SFS}}(\lambda_{\text{ex}}) = Kcl\phi E_{\text{ex}}(\lambda_{\text{ex}})E_{\text{em}}(\lambda_{\text{ex}} + \Delta\lambda)$$

If the selected value for $\Delta\lambda$ is rather small, the SFS line slices through a corner of the TL plot, which results in an SFS spectrum that covers no more than a few nanometers. Fluorescence spectra can sometimes be reduced to a single narrow band if $\Delta\lambda$ is chosen to match the Stokes' shift of the analyte in the particular solvent (for PAHs typically 3–6 nm [14]). The wavelength offset $\Delta\lambda$ cannot be chosen too small because of Rayleigh scattering at $\lambda_{\text{em}} = \lambda_{\text{ex}}$. Obviously, spectral reduction leads to loss of information, but is at the same time one of the main advantages of the SFS method. Reduction of spectral overlap allows the determination of individual PAHs in complex samples, as was demonstrated by Vo-Dinh and Martinez [15].

Especially if the 0–0 band of the excitation and/or emission spectrum is not very intense (e.g. pyrene, BaP), one may prefer to select a larger $\Delta\lambda$ and thus obtain a better sensitivity [15]. In that case, the resulting SFS spectrum will stretch out over a spectral range of approximately $\Delta\lambda$, but may still be much simpler than the conventional fluorescence spectrum. In a conventional emission measurement, the complete spectrum is recorded using the same optimal excitation wavelength. In an SFS measurement, when the wavelength offset is chosen to match the difference between the maxima of excitation and emission, only the emission maximum will be recorded under optimal excitation conditions and with optimal sensitivity. The rest of the spectrum may not be completely removed, but will at least be less efficiently excited. Figure 13.5 presents the conventional and SFS spectra of the PAH metabolite pyrene-1-glucuronide, using $\Delta\lambda = 37$ nm. The absolute intensity of the SFS peak is equal to that of the conventional emission maximum

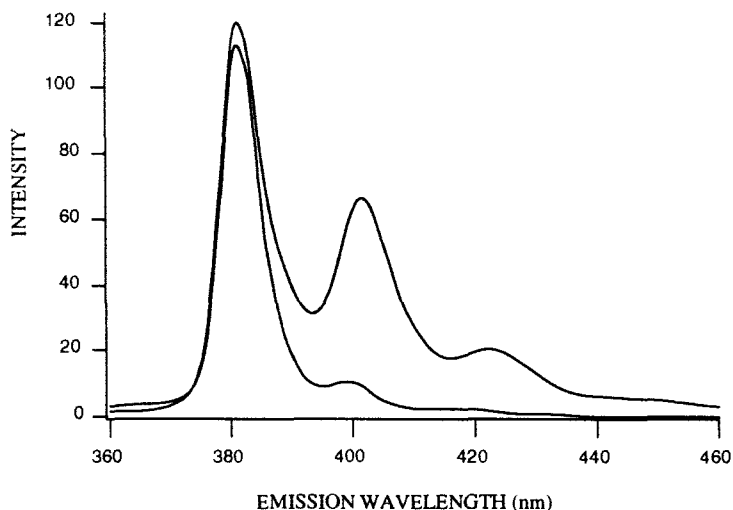


Fig. 13.5. Conventional (top; $\lambda_{\text{ex}} = 345$ nm) and synchronous (bottom; $\Delta\lambda = 37$ nm) fluorescence spectra of 5×10^{-8} M pyrene-1-glucuronide in ethanol/water (50:50); intensities are plotted on the same scale; spectral band passes were 5 nm in both experiments. Reproduced from [2] with permission.

(same excitation and emission maxima, same slit widths), but the spectrum is confined to approximately 37 nm and the intensity of the vibronic emission bands is strongly reduced.

13.3. APPLICATIONS

13.3.1. Shpol'skii spectrofluorimetric analysis of parent PAHs

In this section it is shown that SS is an appropriate analytical technique for the determination of PAHs in environmental analysis. SS was first invoked as an independent identification technique to upgrade routine HPLC analysis of marine sediment samples. Secondly, it was applied independently to the analysis of PAKs in sediment reference materials as a quantitative method and compared with existing techniques, i.e. LC with fluorescence detection and GC-MS. Finally, the applicability of SS to the analysis of such complex samples as biota containing a large amount of interfering substances (e.g. fatty components) is demonstrated.

13.3.1.1. Identification of eluting LC peaks

Gradient LC combined with fluorescence detection is routinely used in the Dutch Water Quality Survey to determine the PAH contents of marine sediment and suspended matter samples. Figure 13.6 shows a typical chromatogram utilizing a gradient elution program starting with water/methanol (30:70) and ending at 100% methanol. Benzo[b]-

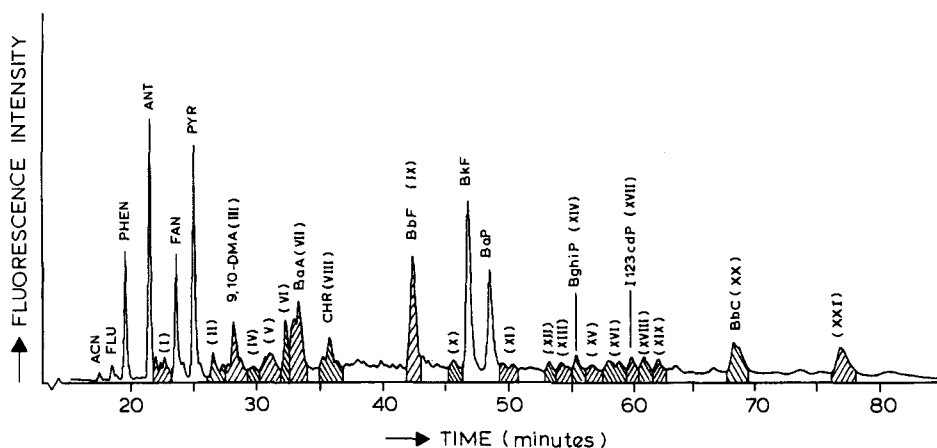


Fig. 13.6. Reversed-phase HPLC chromatogram of suspended matter sample; ACN, acenaphthene; FLU, fluorene; PHEN, phenanthrene; ANT, anthracene; FAN, fluoranthene; PYR, pyrene; 9,10-DMA, 9,10-dimethylanthracene; BaA, benz[a]anthracene; CHR, chrysene; BbF, benzo[b]-fluoranthene; BkF, benzo[k]fluoranthene; BaP, benzo[a]pyrene; BghiP, benzo[ghi]perylene; I123cdP, indeno [1,2,3-cd] pyrene; BbC, benzo[b]chrysene. Reproduced from [16] with permission.

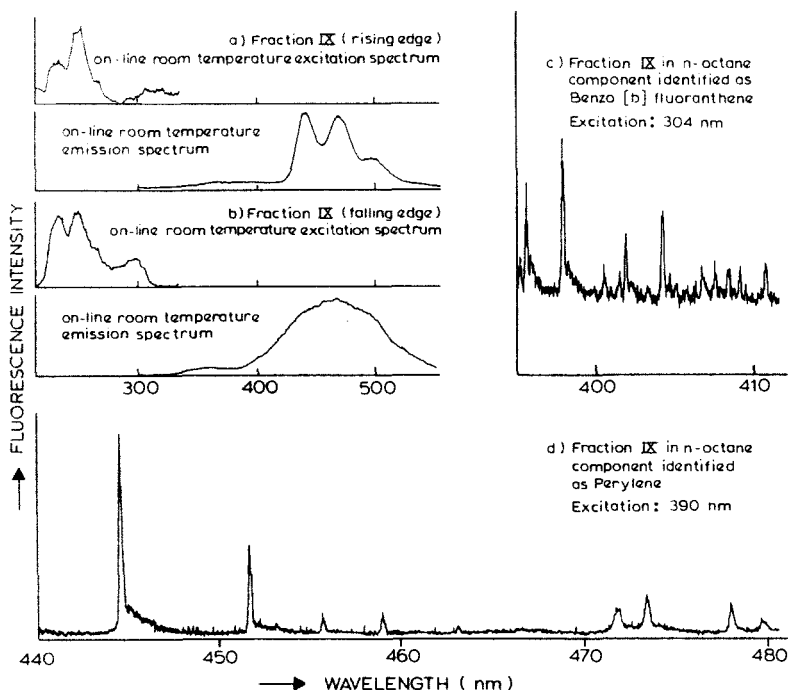


Fig. 13.7. On-line room temperature spectra (a,b) and off-line Shpol'skii spectra (c,d) of fraction IX (Shpol'skii matrix *n*-octane). Reproduced from [16] with permission.

chrysene was added as an internal standard as recommended by the Marine Chemistry Working Group of the International Council for the Exploration of the Sea (ICES). To ascertain the identity and the purity of the peaks in the chromatogram, 21 fractions were collected and analyzed by SS [16]; the PAHs identified prior to Shpol'skii analysis are indicated in Fig. 13.6.

Identification was achieved by comparison with reference Shpol'skii spectra [5,17]. Figure 13.7 shows both the on-line spectra recorded at room temperature and the Shpol'skii spectra of fraction IX. On the basis of retention time, fraction IX was identified as benzo[*b*]fluoranthene (BbF), but the on-line room temperature spectra (recorded at different positions of the eluting peak) indicate that at least one other compound was present. A conclusive identification, however, could not be derived from these spectra. With help of the Shpol'skii spectrum in Fig. 13.7d, the second compound in fraction IX was identified as perylene.

Neither the chromatographic peak shape (Fig. 13.6), nor the room-temperature spectra gave any indication that fraction XV contained more than one component. The Shpol'skii spectra, however, show that not only dibenz[*ah*]anthracene (DBaA) is present, but also some unidentified components (see Fig. 13.8). Between the emission lines of DBaA (marked with an asterisk), several other peaks can be distinguished. The spectra illustrate that, because of the line-narrowing effect obtained with Shpol'skii spectroscopy, even compounds emitting in the same wavelength region will seldom show spectral overlap.

The composition of fraction XX is of special importance, since it contains benzo[*b*]chrysene (BbC), added as an internal standard. Obviously, it is crucial to measure such a standard without interferences. The high-resolution spectra, obtained from fraction XX, are shown in Fig. 13.9, together with a reference spectrum of BbC. Fraction XX does not only contain BbC but at least three other species are present. Unfortunately, only one could be identified, i.e. anthanthrene (ATT); for the two other species, no reference spectra were found. We conclude that one should be careful applying BbC as an internal standard for HPLC/fluorescence purposes, as it will probably be impossible to remove all interferences spectroscopically.

The results of the qualitative analysis of the 21 fractions [16] are summarized in Table 13.1; the number of identified compounds is roughly doubled by invoking SS. Compounds identified in the present study are underlined; an asterisk indicates that a Shpol'skii spectrum was obtained but that no matching reference spectrum was available. The latter aspect underlines the potential of the method; once a larger library of reference spectra is available, the identification possibilities of the Shpol'skii method will be greatly enhanced.

An obvious disadvantage of the Shpol'skii technique is that it can only be practised in an off-line mode. It should be emphasized, however, that the analysis of the separate elut-

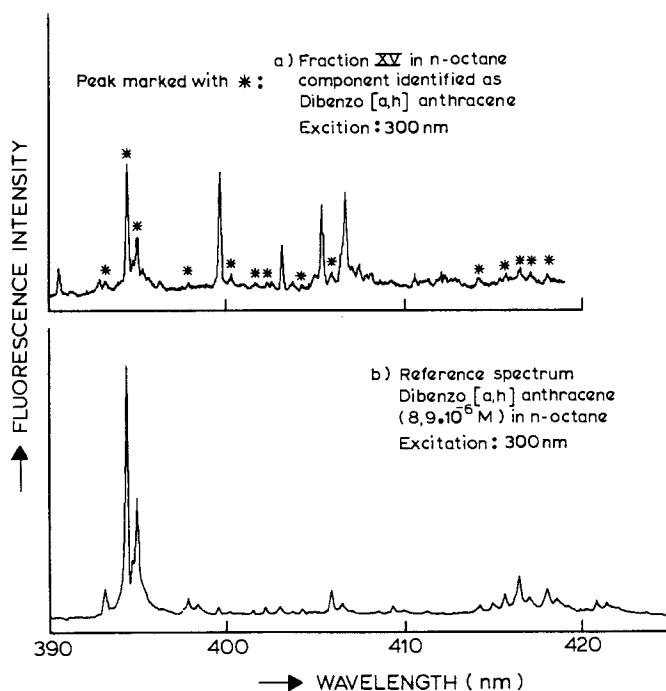


Fig. 13.8. Shpol'skii spectra of (a) fraction XV in *n*-octane and (b) dibenz[*ah*]anthracene standard (8.9×10^{-6} M). The peaks marked with * are attributed to DBaH. Reproduced from [16] with permission.

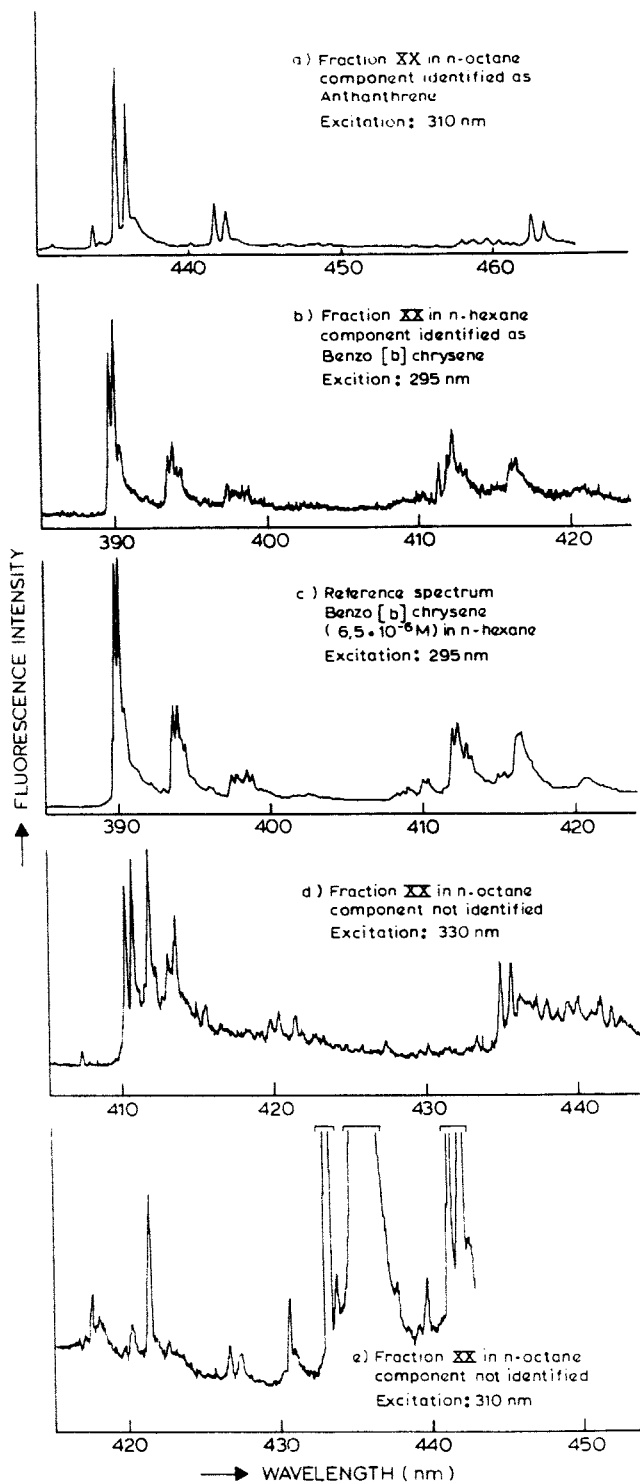


TABLE 13.1

POLYCYCLIC AROMATIC HYDROCARBONS IN SUSPENDED MATTER SAMPLE IDENTIFIED WITH SHPOL'SKII SPECTROSCOPY (FROM [16])

Fraction	PAH	Fraction	PAH
I	<u>4,5-Methylenepheneanthrene</u>	XIII	*
II	<u>Triphenylene</u>	XIV	Benzo[ghi]perylene
V	*	XV	<u>Dibenz[ah]anthracene</u> ; *, *
VI	<u>Benzo[b]fluorene</u>	XVI	*
VII	Benz[a]anthracene; <u>2-methylpyrene</u> ; *	XVII	Indeno[1,2,3-cd]pyrene
VIII	Chrysene; *	XVIII	*, *
IX	Benzo[b]fluoranthene; <u>perylene</u>	XIX	*
X	<u>Dibenz[ac]anthracene</u> ; *, *	XX	Benzo[b]chrysene; <u>anthanthrene</u> ; *, *
XI	<u>Dibenz[a]anthracene</u> ; *, *	XXI	Dibenzo[bk]fluoranthene; *

*Compound showed quasilinear spectrum, but could not be identified. Compounds underlined were identified in this study. No Shpol'skii spectra were obtained from fractions III, IV and XII.

ing fractions needs only be carried out once. The results of this procedure can be used for a (qualitative) validation of the routine HPLC-analysis.

13.3.1.2. Analysis of sediment reference materials

Although the advantages of the Shpol'skii method for the unambiguous identification of closely related compounds are generally recognized [16,18], it was believed that specific features of the method would preclude quantitative applications [19]. For some analyte-solvent combinations, the shape and intensity of the quasilinear Shpol'skii spectra may depend rather critically on various experimental parameters, such as matrix purity, cooling rate and sample holder design [5,9]. For most PAHs of the EPA priority pollutant list, however, reproducible Shpol'skii spectra can be obtained in *n*-octane matrices [20], provided the sample holder and cooling regime is designed for instantaneous solidification [9]. If an appropriate internal standard is used, various experimental sources of error, such as solvent evaporation, the presence of air bubbles or cracks in the frozen sample, and variations in sample thickness, optical alignment or excitation energy, are adequately corrected for.

Here, it is shown that SS can be successfully applied to PAH analysis in sediments. Reference material HS-4, originating from a polluted harbor in Nova Scotia, Canada, was obtained from the National Research Council Canada (NRCC) [2]. The crude Soxhlet extract, containing the PAHs at ca. 10^{-6} – 10^{-7} M concentrations, was diluted 1:1 with a 2×10^{-7} M solution of perdeuterated pyrene (internal standard) in *n*-octane. Hexane was

Fig. 13.9. Shpol'skii spectra of fraction XX (5a, d, e in *n*-octane; 5b in *n*-hexane) and of benzo[b]chrysene standard solution (6.5×10^{-6} M in *n*-hexane) (5c). The strong emission lines in 5e are attributed to anthanthrene. Reproduced from [16] with permission.

carefully evaporated in a gentle stream of nitrogen and replaced with *n*-octane, a suitable matrix for most PAHs with 4–6 fused rings [5]. Samples were cooled to 26 K and analyzed using lamp-excited Shpol'skii spectroscopy.

Peak areas were divided by that of the internal standard and compared to previously determined calibration plots. In order to correct for matrix transmission losses in crude extracts (excitation light and emission from the analyte may be absorbed by the matrix, not necessarily to the same extent as for the internal standard), an absorption spectrum of the extract was recorded. The actual fluorescence intensity I , compared to the intensity I_n that would be observed in a transparent *n*-octane matrix, can be calculated from the matrix absorptions $A_{\lambda_{\text{ex}}}$, $A_{\lambda_{\text{em}}}$ at the excitation and emission wavelength of each compound:

$$\frac{I}{I_n} = \frac{1 - 10^{-(A_{\lambda_{\text{ex}}} + A_{\lambda_{\text{em}}})}}{(A_{\lambda_{\text{ex}}} + A_{\lambda_{\text{em}}}) \ln 10}$$

(front-face geometry; see [21]). In practice, absorption of emission light was negligible; correction factors for absorption of excitation light were typically not larger than 10–20% for the PAHs analyzed in these samples.

The HS-4 extract was not only analyzed using calibration graphs, a standard addition approach was also employed: a synthetic mixture, containing all PAHs to be determined at equal concentrations, was added to the sample at 9×10^{-8} , 3×10^{-7} and 9×10^{-7} M. The internal standard concentration was 1×10^{-7} M in all solutions. The analyte concentration in the original sample was calculated from the intercept, using simple linear regression. The results collected in Table 13.2 show excellent agreement between the two methods, indicating that the calibration curves, determined in clear standard solutions, are also applicable to crude sediment extracts. After correction for matrix absorption, the slope of the calibration plots did not differ from that of the standard addition plots for the

TABLE 13.2

PAH CONCENTRATIONS (mg/g) IN SEDIMENT REFERENCE MATERIAL HS-4

PAH ^a	HPLC-Flu ^b	Shpol'skii; calib. ^c	Shpol'skii; st. add.	NRCC values ^d
Pyr	0.69 ± 0.06	0.91 ± 0.04	0.88	0.94 ± 0.12
BaA	0.39 ± 0.02	0.44	0.46	0.53 ± 0.05
Chr	0.48 ± 0.09	0.38	0.39	0.65 ± 0.08
BaP	0.43 ± 0.04	0.53 ± 0.02	0.49	0.65 ± 0.08
BbF	0.48 ± 0.04	0.68	0.66	0.70 ± 0.15
BkF	0.28 ± 0.03	0.35 ± 0.03	0.33	0.36 ± 0.05
BgP	0.37 ± 0.06	0.40	0.39	0.58 ± 0.22

^aPyr, pyrene; BaA, benz[*a*]anthracene; Chr, chrysene; BaP, benzo[*a*]pyrene; BbF, benzo[*b*]fluoranthene; BkF, benzo[*k*]fluoranthene; BgP, benzo[*ghi*]perylene.

^bResults of 3 determinations.

^cResults of 1–4 determinations.

^dUncertainties represent 90% confidence limits.

compounds listed in Table 13.2. Since the standard addition method requires several measurements for each sample, the calibration graph method was obviously preferred.

The data collected in Table 13.2 are within or just below the 90% confidence interval stated by the NRCC except for chrysene. There seems to be a slight negative bias, which could be the result of incomplete extraction. The HPLC-fluorescence results, obtained at the Tidal Waters Division using the same extracts, also agree reasonably well with the reference values, although the negative bias is more pronounced.

Summarizing, we conclude that lamp-excited Shpol'skii spectrometry is a suitable technique for the quantitative analysis of a range of PAHs. Measurements could be carried out on crude Soxhlet extracts without further clean-up, thus reducing sample handling time and the risk of introducing contamination. As a rule, "true" PAH concentrations in reference materials are determined by employing a spectrum of different analytical techniques. Since the selectivity of the Shpol'skii technique is based on spectral rather than physical separation, it is strongly recommended to include this fully independent method in quality control procedures.

13.3.1.3. Analysis of biota

The analysis of PAHs in biota is more difficult than in sediment samples, owing to the low concentrations of the analytes and the high levels of potentially interfering substances. Nevertheless, using the Shpol'skii technique, a number of PAHs could be determined in a complex biotic extract without prior chromatographic separation [21]. The bioaccumulation of PAHs in living organisms is used to obtain measurable and time-integrated levels of the total biologically available fraction of these compounds in the area. The latter method is called active biological monitoring, and mussels have been demonstrated to be particularly useful for this purpose ("mussel watch"; [22]). A mussel constantly pumps and filters large amounts of sea water (ca. 50 l/day), and PAHs accumulate in the fatty tissues until the uptake is balanced by excretion and degradation and a plateau value is reached. Also, detection of PAHs in other biota tissues is of interest for biologists and environmental chemists.

After removing the shell, mussels were homogenized, freeze-dried, extracted with *n*-hexane in a Soxhlet apparatus and evaporated to dryness. Terns were plucked, freeze-dried, homogenized, freeze-dried again, extracted with hexane and evaporated to dryness. Of the fatty, brownish samples thus obtained, half was simply diluted with *n*-octane (Baker analyzed grade) and measured without further clean-up. The remaining part of the extracts was further cleaned over a silica gel column (15 g, deactivated with 5% water), and eluted with hexane. For the tern sample, this washing procedure had to be repeated several times in order to obtain a sufficiently clear extract. Before measurement, a known concentration of internal standard, perdeuterated pyrene, was added, and hexane was replaced with octane through selective evaporation in a stream of nitrogen. Attempts to obtain Shpol'skii spectra directly with the concentrated fatty extracts were unsuccessful; optimum results were obtained after diluting the sample 100-fold with octane. Although the background noise was not much higher than for neat academic solutions, the detection limits were of course greatly affected by the necessity of dilution. Nevertheless, it was still possible to determine BaP, BkF and pyrene in both samples [21]; the results are

TABLE 13.3

DETERMINATION OF PAHs IN CRUDE AND NEAT SAMPLES^a

PAH	Tern before clean-up	Tern after clean-up	Mussel before clean-up	Mussel after clean-up
Benz[<i>a</i>]anthracene		8		40
Benzo[<i>a</i>]pyrene	9 (10)	4	27	17
Benzo[<i>ghi</i>]perylene		17		22
Benzo[<i>k</i>]fluoranthene	11 (11)	7	36	18
Chrysene		13		76
Pyrene	156 (153)	143	256	265
Perylene		2		8

^aValues are in ng/g organism; data in parentheses were measured using standard addition.

summarized in Table 13.3. Figure 13.10a shows part of the tern emission spectrum, exhibiting the main fluorescence peaks of pyrene and the internal standard.

One could imagine that the Shpol'skii analysis of biota samples would benefit from a sample clean-up. Using this procedure, the fatty components were largely removed and the samples became virtually transparent in the visible and near-UV regions. Strong dilution was no longer necessary, and much larger signals could be obtained in this way. This is illustrated by the spectrum for the cleaned tern sample in Fig. 13.10b, as compared to that of the crude sample in Fig. 13.10a. The samples were checked for ten different PAHs,

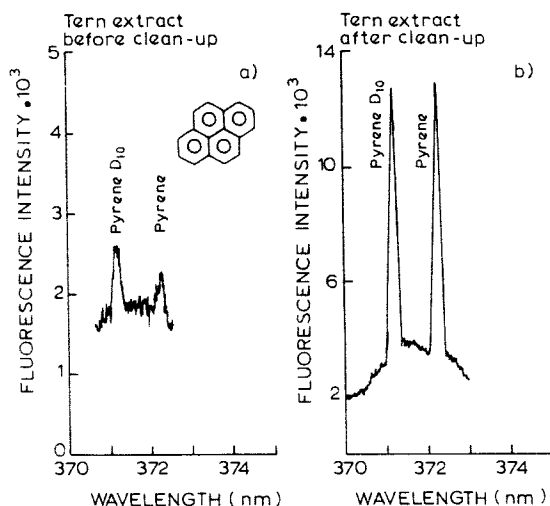


Fig. 13.10. Part of the Shpol'skii fluorescence spectra of the tern sample; lamp excitation, 335 nm; temperature, 26 K. (a) Before clean-up; [pyrene-*d*₁₀] = 3×10^{-8} M; (b) after clean-up; [pyrene-*d*₁₀] = 1×10^{-7} M. Reproduced from [21] with permission.

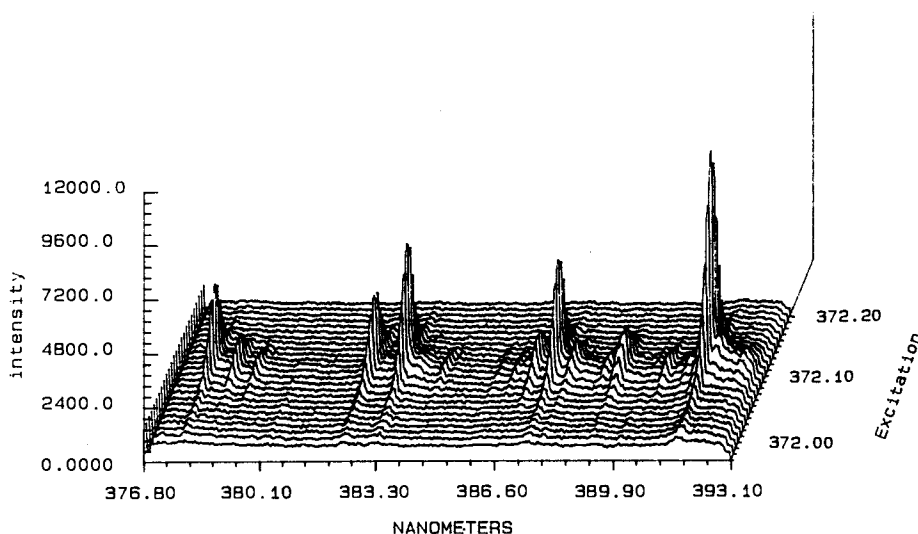


Fig. 13.11. Excitation-emission plot of pyrene in *n*-octane at 28 K; conc. = 5.0×10^{-7} M, laser excitation. Reproduced from [23] with permission.

of which seven could be determined (see Table 13.3). Benzo[*b*]fluoranthene, benzo[*e*]-pyrene and indeno[1,2,3-*cd*]pyrene could not be detected. For pyrene, the standard deviation was found to be 4.7% over seven independent measurements. For all compounds, the repeatability was better than 10%.

The results indicate that SS is appropriate for the direct determination of PAHs in fatty biotic samples. Fatty acids and other kinds of non-polar compounds hardly interfere with the measurements, as long as their total content is not much larger than 1% after dilution with octane. Of course, the dilution step leads to a severe decrease in sensitivity, but nevertheless three different aromatic compounds could be detected at the ng/ml level in the crude extract. The detection potential can be expected to be considerably improved if a laser is used for excitation instead of a xenon arc lamp, in other words by applying laser excited Shpol'skii spectroscopy (LESS).

Depending on the laser system applied, there are three important advantages compared to lamp excitation [23]:

- (1) High excitation power: compared to the light intensity of a xenon arc lamp dispersed by a high-throughput monochromator, a Nd:YAG/dye laser combination may offer an increase in excitation power of typically two orders of magnitude. Furthermore, the laser beam can easily be focussed on a very small (microliter) sample volume. This is important, as in high-resolution spectroscopy, the fluorescent spot has to be projected onto the very narrow entrance slit of the emission monochromator. Overall, we can obtain a total increase in the effective excitation power of three orders of magnitude.
- (2) High monochromaticity: since the S_1 - S_0 region of the Shpol'skii absorption spectrum is also narrow-banded [5], it is possible to selectively excite one particular compound in a mixture. Figure 13.11 shows a three-dimensional excitation-emission spectrum of pyrene in *n*-octane [2]. Emission spectra were continuously recorded as the laser

was tuned through the 0–0 transition. Pyrene- d_{10} was also present in the mixture, but although its excitation wavelength is shifted over only one nanometer, it is completely invisible, as the laser line does not match exactly. It is clear that we have here a powerful tool to increase the emission of a particular analyte and at the same time reduce interferences from other compounds. Figure 13.12a shows the pyrene emission from the crude tern extract using laser excitation.

- (3) Time-resolved detection: if the laser system is of the pulsed type, we can discriminate between the relatively long-living (20–500 ns) emission of the aromatic analytes and instantaneous processes like stray-light or (Raman) scattering. Also short-living

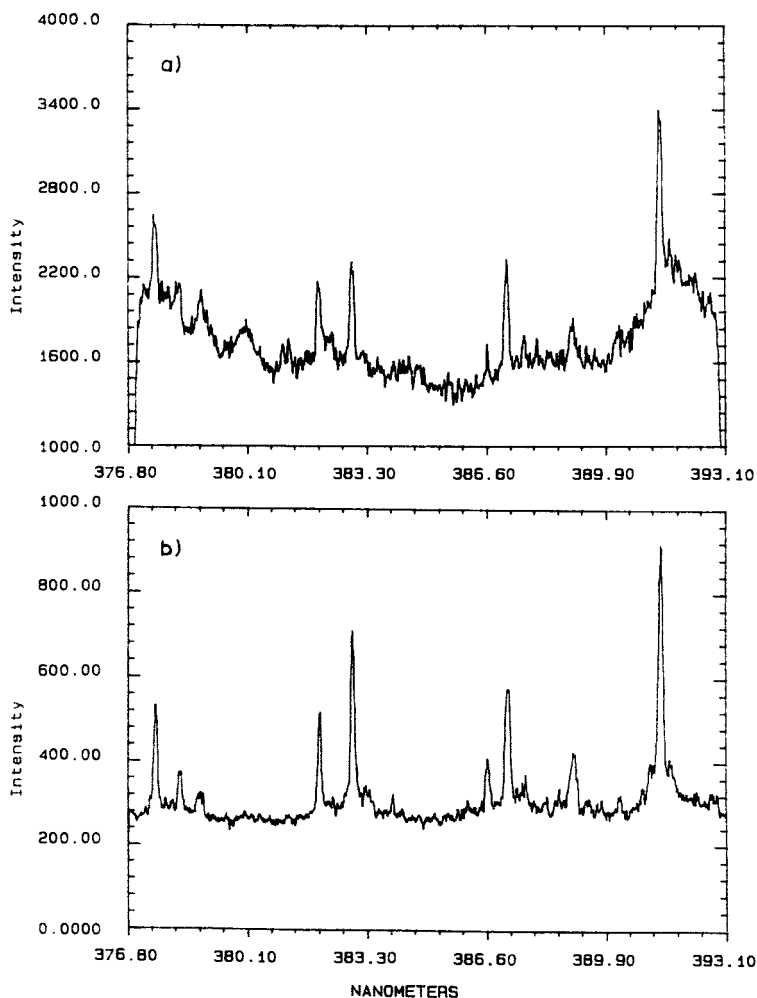


Fig. 13.12. Shpol'skii emission of pyrene in crude tern extract in *n*-octane at 28 K; laser excitation 372.10 nm. (a) No time resolution; (b) with time-resolved detection (delay = 50 ns). Reproduced from [23] with permission.

background luminescence is removed. A pulse generator was used to activate the photocathode of the diode-array detector 50 ns after the laser shot. The effect of time-resolution is illustrated if we compare the signal-to-noise ratios in Figs. 13.12a,b. Of course, for shorter-living analytes a shorter delay must be used and the background noise may not be removed completely.

The overall spectral improvement due to laser excitation is clearly demonstrated if we compare Fig. 13.12b to Fig. 13.10a, showing the pyrene emission from the same crude tern extract. The advantages are obvious: both sensitivity as well as selectivity are remarkably improved.

To summarize, SS can be used to qualitatively and quantitatively determine PAHs in environmental samples [2,20]. The technique is very sensitive: the limit of detection for pyrene (in a synthetic mixture of 10 PAHs in *n*-octane using conventional lamp excitation) is as low as 5×10^{-10} M and for BaP with laser excitation a detection limit of 5×10^{-12} M has been obtained (for a 10- μ l sample volume this means 50 amol [2]). For trace level analysis of PAHs in complex samples, it will be essential to employ laser excitation. Of course, one should realise that laser systems offering acceptable powers in the near-UV region are still quite expensive, and an extensive wavelength range cannot be reached without having to change the dye solution or the system configuration. Nevertheless, for a number of complicated analytical problems, (laser excited) Shpol'skii spectroscopy will offer an adequate solution.

13.3.2. Shpol'skii spectrofluorimetric analysis of PAH metabolites

In order to monitor exposure to PAHs in the environment, the determination of their concentrations in the various environmental compartments may not be sufficient, since bioavailability is not taken into account. Biological monitoring, that is, the determination of a particular compound (or its metabolites) in a specific organism or tissue, can provide valuable information on the actual uptake rate [24]. Fish usually do not show considerable accumulation of PAHs [25]. Upon absorption, PAHs are rapidly metabolized into more polar derivatives that are stored in the gallbladder to be excreted [26,27]. Attempts to biomonitor PAH uptake by fish should therefore concentrate on PAH metabolites in excreta rather than on parent PAHs in tissue. Krahn and co-workers [28] developed HPLC/fluorescence and GC-MS methods to determine PAH metabolites in fish bile.

The potent carcinogen BaP is often used as a model compound to study the toxic effects of PAHs [29]. BaP is metabolized by hepatic enzyme systems into a number of mono- and polyhydroxylated derivatives. Some reactive species may form adducts with proteins or DNA, but most metabolites are rapidly excreted in the form of glucuronide, sulfate, or glutathion conjugates [30]. In laboratory experiments, the biotransformation products of BaP are usually analyzed by means of HPLC; to avoid problems with detection sensitivity, toxicologists can administer high doses of BaP or use radioactive material. In the field, the concentration of BaP metabolites in bile of feral fish could be used as an indicator of exposure to BaP and related PAHs in the area, but the detection of BaP metabolites requires extremely sensitive and selective methods. Bile of fish exposed to many different PAHs will contain an even more complex mixture of PAH metabolites that may interfere with the analysis. Using HPLC/fluorescence, Krahn and colleagues [31]

succeeded in detecting 3-hydroxy-BaP (3-OH-BaP) in some bile samples from a highly polluted site near Seattle, but sub-ppb sensitivity would be needed for the detection of BaP exposure in other, less polluted areas. As an extra complication, the bile volumes available are usually not sufficient for trace enrichment.

Shpol'skii spectrometry may offer the required sensitivity and selectivity, but PAH metabolites will be less compatible with the matrix than their parent compounds because of their increased polarity. Weeks and co-workers [32] described a procedure to transform monohydroxy-benz[*a*]anthracenes into less polar methoxy derivatives, which could subsequently be analyzed by means of LESS. Recently, the same research group reported the derivatization and Shpol'skii spectra of a wide range of BaP metabolites: monohydroxy-BaP derivatives, BaP-dihydrodiols, BaP-dihydrodiolepoxide, as well as BaP-tetrahydro-tetrol [33].

The practical applicability of the Shpol'skii technique to the analysis of BaP metabolites in fish bile has been shown in the literature [2,34]. An analytical protocol was developed for the quantitation of 3-OH-BaP in bile samples. The model fish studied was the flatfish species flounder (*Platichthys flesus*). Exposure to BaP was realized following two different methods. High exposure levels were realized by administering a single dose of BaP (parenteral injection in acetone/Mulgofen 620; 0.78 or 4.04 mg/kg body weight). The fish were fed shrimp (*Crangon crangon*) until 2 days before injection and were sacrificed 48 h after injection. To simulate semi-chronic exposure to realistic BaP pollution levels, flounders were kept during 4 weeks in three different mesocosms: (1) moderately polluted Rotterdam harbor sediment (dredging class II, direct contact with the sediment was possible); (2) indirect exposure to Rotterdam harbor sediment (Wadden Sea sand bottom; food and water equilibrated with the polluted harbor sediment); (3) Wadden Sea sand bottom (control group). The PAH contents of the sediments (fine fraction only) were determined after wet sieving over a 63 μm nylon filter, by means of HPLC with fluorescence detection [35]. The harbor sediment contained 450 ppb BaP and 800 ppb pyrene; the Wadden Sea sand contained 140 ppb BaP and 180 ppb pyrene (ng/g dry weight of fine fraction). The fish were fed until 2 days before section to allow the accumulation of metabolites in the gallbladder and to reduce the confounding effects from different feeding habits.

Bile was collected from the gall bladder by means of a syringe and stored in vials in the dark at -20°C until further use. The bile samples were treated as follows: 20 μl of bile was diluted with water to 1 ml and incubated for 2 h at 37°C with 20 μl of β -glucuronidase/aryl sulfatase solution to hydrolyze conjugated metabolites. Typically, maximum yield was reached within 20–30 min. The free metabolites were quantitatively extracted by repeated extractions with *n*-hexane (4 times 3 ml). For direct analysis of underivatized metabolites, hexane was evaporated in a stream of nitrogen and the residue dissolved in 2 ml of *n*-octane. In most cases, however, the volume of the extract was reduced to ca. 0.5 ml and the metabolites derivatized according to a procedure adopted from Weeks and co-workers [32]: 2 mg of sodium hydride was washed three times with *n*-pentane in a flask under nitrogen atmosphere, 1 ml of dimethyl sulfoxide (DMSO) was added and the mixture was stirred at 70°C for several minutes until the formation of H_2 bubbles had ceased. After cooling to room temperature, 100 μl of methyl iodide and the bile extract were added; after several minutes of stirring the reaction was quenched with 4 ml of

water. The methylated products were quantitatively extracted with 2 times 3 ml of *n*-hexane. This extraction solvent was preferred over *n*-octane because of its lower boiling point and higher purity. For Shpol'skii analysis, the extract was concentrated and the solvent gradually replaced with *n*-octane in a gentle stream of nitrogen. For quantitation, perdeuterated perylene was added to the final analytical sample as an internal standard; 2×10^{-8} M for bile samples from the most polluted mesocosm, 2×10^{-9} M for the other samples.

Direct Shpol'skii analysis of monohydroxy-BaP metabolites is possible; 1-OH-BaP and 3 OH-BaP were identified in fish bile after injection with BaP [36]. However, the sensitivity of the method proved insufficient for application to the mesocosm study. These analytes are not fully compatible with the crystalline *n*-octane host. As a consequence, the actual concentration of analyte molecules trapped in crystalline sites and producing quasilinear emission is rather low and thus the achievable sensitivity is disappointing. Furthermore, the shape and intensity of the Shpol'skii spectra depend critically on concentration, cooling rate or the presence of polar impurities in the matrix, so proper quantitation in real samples is very difficult [37].

It has been observed that for phenolic metabolites, a derivatization reaction with methyl iodide in DMSO is appropriate to solve these problems [34]: the reaction is very rapid, practical, straightforward and quantitative, and the methylated analytes provide good Shpol'skii spectra in *n*-octane with the possibility of isomer-specific determination (see Fig. 13.13). To illustrate the increase in sensitivity: in case of 3-OH BaP, the improved host-guest compatibility after derivatization resulted in a 20-fold increase in quasilinear fluorescence intensity; the detection limit improved to 5.0×10^{-10} M (lamp excitation at 300 nm).

A typical Shpol'skii spectrum of a methylated bile extract (originating from fish that had received a high dose via injection) is shown in Fig. 13.14. Non-selective laser excitation at 348 nm was employed, in order to be able to determine all metabolites simultaneously; the overall dilution factor was 1000. The spectrum is dominated by 3-methoxy BaP and 1-methoxy BaP (compare with reference spectra in Fig. 13.13c and a); the relative contribution of the latter varied considerably between individuals: between 7 and 26% of the total amount of metabolites detected. The metabolite 3-OH BaP was chosen as a marker compound for the biomonitoring of BaP uptake from a polluted environment.

For quantitation of 3-methoxy BaP with LESS, an internal standard must be added to the analytical sample to compensate for variations in sample thickness, laser power and optical alignment. In order to assure proper correction for laser output fluctuations (meanwhile saving time as well), the internal standard should be excitable at the wavelength chosen for the analyte, and should have a sufficiently strong emission line in the emission window covered by the multichannel detector. Perdeuterated perylene was found to meet the above requirements; ratioing the peak areas of the 0-0 emission lines, a straight calibration curve was obtained for 3-methoxy BaP in the concentration range of interest (3×10^{-11} M to 1×10^{-8} M).

The absolute detection limit ($S/N = 3$) for 3-methoxy-BaP, using laser excitation at 418.36 nm, was found to be 5×10^{-12} M (50 amol) in *n*-octane solutions. The detection in bile extracts was not seriously affected by matrix interferences. When the sample treatment was carried out without overall dilution (provided that sufficient bile was collected),

the detection limit was still 2×10^{-11} M or 0.005 ng/ml. For most samples, we used an overall dilution factor of 20; in that case the detection limit was 2×10^{-10} M or 0.05 ng/ml in the original sample, which was sufficient to detect exposure to BaP in all samples from the mesocosm experiment. The repeatability of the method (four replicates of sample extraction and determination) was 16%.

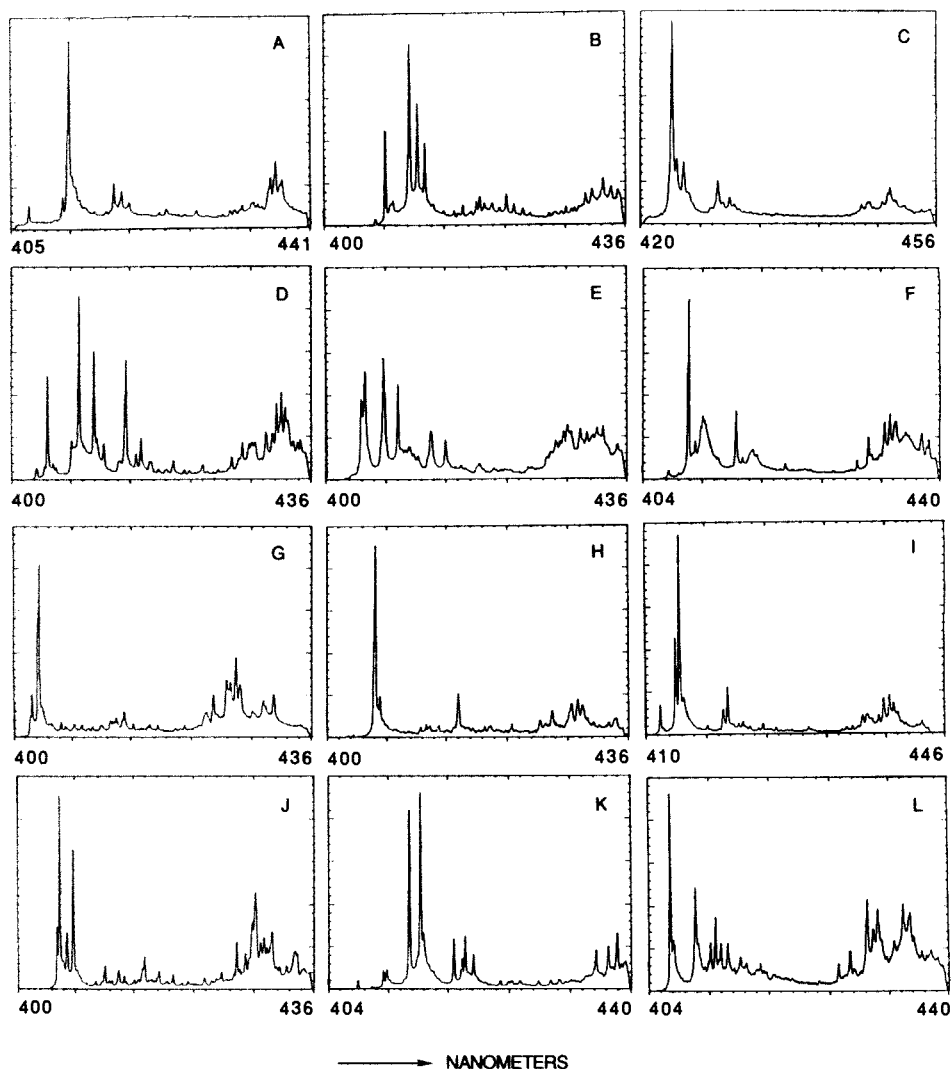


Fig. 13.13. Shpol'skii spectra in *n*-octane of phenolic BaP metabolites after derivatization, using non-selective laser excitation at 348 nm. (A) 1-Methoxy BaP, 10^{-7} M; (B) 2-methoxy BaP, 10^{-6} M; (C) 3-methoxy BaP, 2×10^{-7} M; (D) 4-methoxy BaP, 10^{-6} M; (E) 5-methoxy BaP, 10^{-6} M; (F) 6-methoxy BaP, 10^{-6} M; (G) 7-methoxy BaP, 10^{-6} M; (H) 8-methoxy BaP, 10^{-6} M; (I) 9-methoxy BaP, 10^{-6} M; (J) 10-methoxy BaP, 10^{-6} M; (K) 11-methoxy BaP, 10^{-6} M; (L) 12-methoxy BaP, 10^{-6} M. Reproduced from [34] with permission.

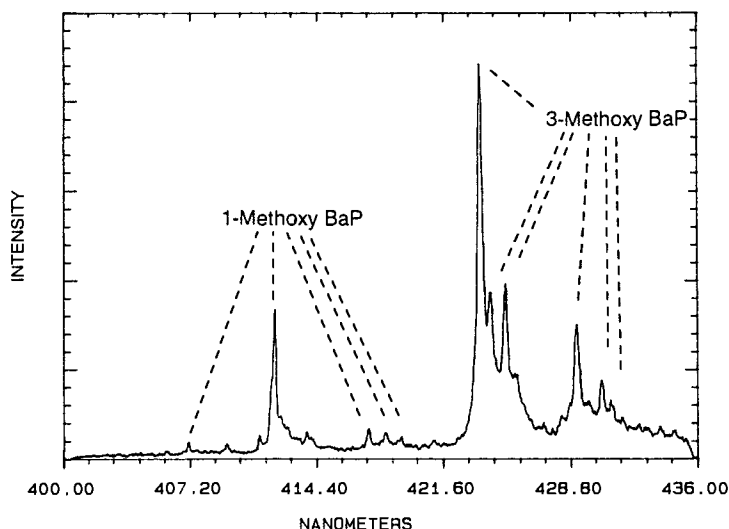


Fig. 13.14. Shpol'skii spectrum of methylated flounder bile sample (0.78 mg/kg BaP injected), featuring 1-methoxy BaP and 3-methoxy BaP. Non-selective laser excitation at 348 nm. Reproduced from [34] with permission.

Laser excited Shpol'skii spectrometry of 3-OH-BaP was applied to a mesocosm study in which flounders were exposed during 4 weeks to three degrees of pollution. Mesocosms 1 and 3 reflect the range of PAH pollution levels encountered in the Dutch coastal waters and estuaries. Mesocosm 2 was designed to find out what route of exposure contributes most significantly to the total BaP uptake. Hydrolyzed bile samples were derivatized, the methylated extracts were cooled to 23 K and their Shpol'skii spectra recorded using selective laser excitation at 418.36 nm. For the mesocosm samples, non-selective excitation at 348 nm could not be used because of two reasons: limited sensitivity and spectral overlap with emission bands from 1-methoxy-pyrene (1-OH-pyrene is usually present at much higher levels in fish bile). Notwithstanding the 20-fold dilution caused by the sample workup, 3-methoxy-BaP could be detected in all samples, even from mesocosm 3 (Fig. 13.15). The multiplet structure from Fig. 13.13c has disappeared as the result of site-selective excitation. The analytical results, summarized in Table 13.4, show that fish exposed to Rotterdam harbor sediment had absorbed and metabolized 40 times more BaP than fish from the Wadden Sea sand basin. Furthermore, fish from the second mesocosm showed only a six-fold increase, indicating that some uptake of BaP can take place through the water phase or through the diet [38], but that direct contact with the sediment is the major route of exposure for a bottom-dwelling fish like flounder. Direct absorption through skin or gills, or ingestion of PAH-containing particles, may both be important factors.

Table 13.4 also lists 1-hydroxypyrene levels determined by means of synchronous fluorescence spectrometry (SFS) [39]. Although 1-methoxypyrene can be measured with Shpol'skii spectrometry in an *n*-octane matrix [40], the relatively high concentrations al-

lowed the determination (in a large number of samples) with a faster, more conventional method as will be shown in the next section. The 1-OH-pyrene data show a similar trend as the results for 3-OH-BaP, but the 1-OH-pyrene concentrations are a factor of 300–600 higher, which is not explained by the relative contents of the parent PAHs in the sediments, nor by the fact that 3-OH-BaP is not the only metabolite of BaP. Apparently, the bioavailability of pyrene is much higher than that of BaP, which agrees with the kinetic studies of Landrum [24]. The standard deviations reported in Table 13.4 are an indication of the usual biological spread [26].

It was concluded that the described procedure is appropriate for the quantitative determination of 3-OH-BaP in fish bile. Employing enzymatic hydrolysis, chemical derivatization, and laser excited Shpol'skii spectrometry, the detection limit is as low as 0.005 ng/ml, which is amply sufficient for the biomonitoring of BaP uptake in the Dutch coastal waters and in inshore areas. Some extra effort will be required to monitor the much lower levels of BaP pollution at open sea.

13.3.3. Synchronous fluorescence spectrometry of PAH metabolites

In the preceding section, it was shown that direct Shpol'skii analysis of monohydroxy-BaP metabolites in fish bile is possible if chemical derivatization (methylation) is applied and laser- instead of lamp excitation is invoked. Evidently, the synchronous fluorescence spectroscopy (SFS) technique is not sensitive enough for the determination of 3-OH-BaP. However, the concentrations of 3-OH-BaP in bile, measured with Shpol'skii spectroscopy, seem to correlate with the 1-hydroxypyrene levels (see Table 13.4). Since 1-OH-py-

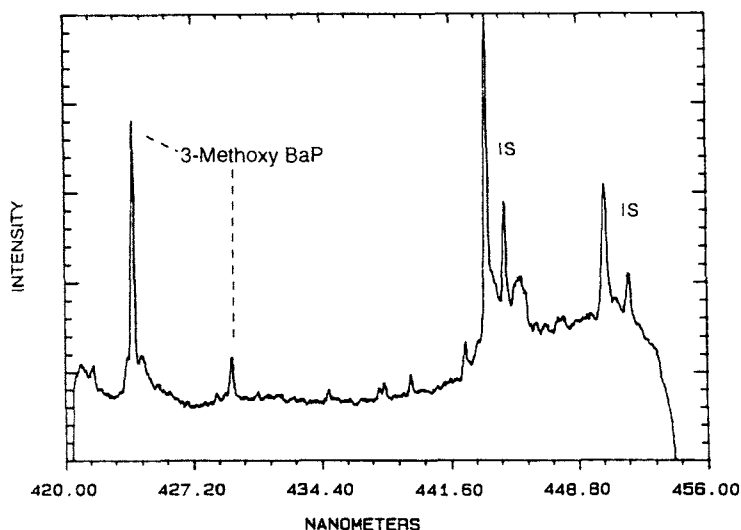


Fig. 13.15. Shpol'skii spectrum of methylated bile extract from Wadden Sea mesocosm, featuring 3-methoxy BaP. Selective laser excitation at 418.36 nm; IS, perylene- d_{12} . Reproduced from [34] with permission.

TABLE 13.4

BaP AND PYRENE METABOLITES IN FISH BILE AFTER EXPOSURE TO DIFFERENT MESOCOSMS [2,34]^a

		3-OH-BaP (LESS)	1-OH-pyrene (SFS)
1	Harbor sediment (direct contact possible)	50 ± 36 (<i>n</i> = 9)	15900 ± 6700 (<i>n</i> = 23)
2	Harbor sediment indirect (PAH uptake through food and/or water)	7.7 ± 2.4 (<i>n</i> = 4)	2600 ± 1500 (<i>n</i> = 16)
3	Sand bottom	1.2 ± 0.1 (<i>n</i> = 3)	800 ± 480 (<i>n</i> = 26)

^aConcentrations, in ng/ml, are expressed as the arithmetic mean ± standard deviation (number of samples).

rene is a major metabolite in bile of fish exposed to PAH polluted sediments [31], it is interesting to examine the applicability of SFS as a rapid screening technique for the determination of this compound [39].

The HPLC-fluorescence chromatogram of hydrolyzed bile from flounder (mesocosm 1, see previous section) is depicted in Fig. 13.16. The compound eluting at 4 min (RP-18 column, acetonitrile/water 70:30 v/v) was 1-OH-pyrene, unambiguously identified by Shpol'skii spectroscopy [40].

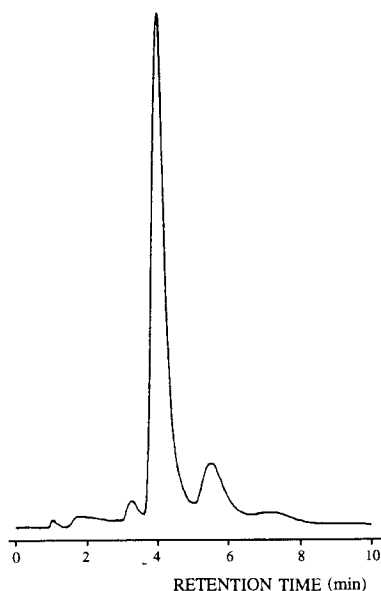


Fig. 13.16. HPLC separation of flounder bile sample from mesocosm 1, after hydrolysis and extraction with *n*-hexane. 1-hydroxypyrene elutes at *t* = 4 min. Fluorescence detection 345/395 nm. Reproduced from [39] with permission.

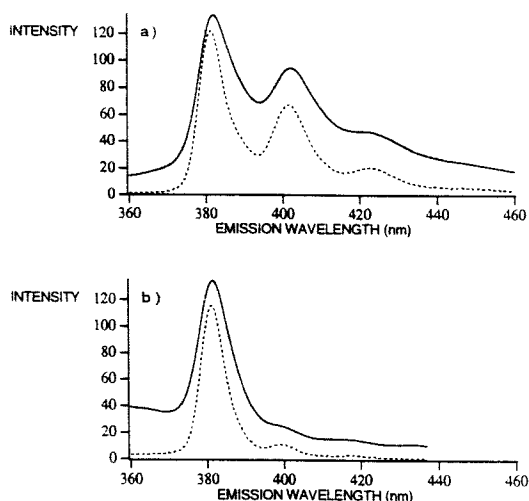


Fig. 13.17. Conventional (a, $\lambda_{\text{ex}} = 345$ nm) and synchronous (b, $\Delta\lambda = 37$ nm) fluorescence spectra in ethanol/water (50:50). Full lines, bile sample from mesocosm 1, diluted 1:2000. Dashed lines, pyrene-1-glucuronide reference standard; 5×10^{-8} M. Reproduced from [39] with permission.

Figure 13.17a presents a conventional fluorescence emission spectrum of diluted bile of flounder after exposure to Rotterdam harbour sediment, compared to the emission spectrum of a pyrene-1-glucuronide standard solution. The spectral features of conjugated 1-OH-pyrene are easily discerned in the total bile spectrum, but correct quantitation is hampered by interferences. Figure 13.17b illustrates the advantage of synchronous scanning: the standard spectrum of pyrene-1-glucuronide is almost completely reduced to one single emission band. In the SFS spectrum of total bile, the emission peak attributed to pyrene-1-glucuronide is now more reliably quantitated.

The SFS spectra were recorded with a wavelength interval $\Delta\lambda$ of 37 nm. SFS analysis of parent PAHs in environmental samples is often carried out using a much smaller $\Delta\lambda$ (typically 3–5 nm, corresponding to the compounds Stokes' shift), in order to achieve maximal spectral simplification. The Stokes' shift of conjugated 1-OH-pyrene, however, is too small (only 1.5 nm) and the molar extinction coefficient of the 0–0 absorption band is too low for practical application. Using $\Delta\lambda = 37$ nm, light scattering was strongly reduced and sensitivity was optimal. The repeatability of the method was 9% (six independent determinations of a bile sample from the reference mesocosm). The limit of detection ($S/N = 3$) was 0.1 ppb in the final analytical solution, which corresponds to 50–200 ppb in the original bile sample. The detection limit is directly proportional to the dilution factor, required to obtain a sufficiently transparent matrix. For less deeply colored samples (i.e. in field samples from fish that are not starved prior to section), much lower detection limits can be obtained (10–20 ppb).

13.3.3.1 Calibration

Obviously, the most straightforward way to perform quantitative fluorimetric measurements would be to use a series of pyrene-1-glucuronide standard solutions for calibra-

tion. Unfortunately, this compound is, as far as we know, not commercially available. Another problem is the fact that the conjugate is quite stable at -20°C , but at ambient temperatures hydrolysis was observed. For these reasons, the possibility of using free 1-OH-pyrene as an alternative standard was explored. This approach can be followed: the fluorescence excitation and emission spectra of free and conjugated 1-OH-pyrene are rather similar in shape, but two effects must be taken into account. The spectra of the conjugate are blue shifted by 5 nm and more intense by a factor of 2.2 ± 0.1 ($n = 5$) (see Fig. 13.18). The latter phenomenon can be fully ascribed to a difference in fluorescence quantum yield. Using excimer laser excitation a fluorescence lifetime of 15 ± 2 ns was measured for free 1-hydroxypyrene, compared to 31 ± 2 ns for the pyrene-1-glucuronide [39].

13.3.3.2. Validation of the method

1-OH-pyrene was determined in a number of flounder bile samples using two independent methods: SFS and HPLC-fluorescence. The concentrations ranged from 280 ng/ml (lowest value from mesocosm 3) to 27.300 ng/ml (highest value from mesocosm 1). Quantitation of the SFS intensities was carried out using standard solutions of free 1-OH-pyrene and a correction factor of 2.2. Calibration solutions in 500-fold diluted bile from a reference site yielded equal intensities as calibration solutions in clean ethanol/water, indicating that matrix absorption and other possible quenching effects had been sufficiently reduced by dilution. Chromatographic and spectroscopic techniques yielded comparable results; the concentrations determined with HPLC were on average $93 \pm 17\%$ ($n = 14$) of the values determined with SFS. It is concluded that the determination of 1-hydroxy pyrene with the rapid SFS technique can be carried out with sufficient accuracy. The precision of the method is fully adequate regarding the biological variability encountered in this type of samples.

Recent testing of the method at the North Sea and at coastal and freshwater sites in The Netherlands has revealed that the SFS method is usually sensitive enough for field monitoring purposes. Only at some remote North Sea locations, were 1-OH-pyrene levels close to the detection limit of SFS measured [2]. Thus, provided the PAH metabolite profile is roughly constant, monitoring of 1-OH-pyrene by SFS can be used as an indication of the local exposure levels to (combustion-related) PAHs. Whenever there are reasons to sus-

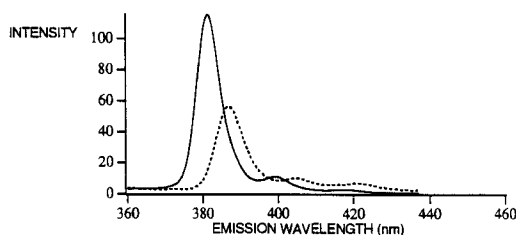


Fig. 13.18. SFS spectra in ethanol/water (50:50); $\Delta\lambda = 37$ nm. Full line, pyrene-1-glucuronide conjugate before hydrolysis. Dashed line, 1-hydroxy pyrene (hydrolysis product). Reproduced from [39] with permission.

pect a gross deviation from the usual metabolite profile (for instance in the case of exposure to creosote or petroleum), then more specific techniques like HPLC-fluorescence, GC-MS or laser excited Shpol'skii spectrometry could be used to determine the relative contribution of other PAH metabolites.

13.4. CONCLUSIONS

Shpol'skii spectroscopy of parent PAHs has shown to be applicable to various types of samples of ecotoxicological interest, for example sediments or biota extracts. Because of the excellent identification capacities of the technique, it can be used in a qualitative way for the identification of unknown compounds and for the assessment of HPLC peak purity. Carrying out such a procedure once for each type of sample would greatly improve the quality of routine HPLC measurements. Considering the potential of Shpol'skii spectrometry as a quantitative analytical technique, a point questioned in the literature, it has been shown [2] that accuracy and precision of the method are fully adequate if care is taken to ensure reproducible sample preparation and cooling procedures, and if a proper internal standard is employed. The application of Shpol'skii spectrometry as an extra independent analytical technique will be especially useful in case large discrepancies are observed between analytical results and references values or between analytical results obtained with different (chromatographic) methods.

Conventional Shpol'skii spectrometry can also be employed to the biological monitoring of PAH exposure (accumulation of parent PAHs), using an extraction and cleanup procedure equal to that routinely applied for HPLC analysis. It was demonstrated that Shpol'skii analysis in crude, lipid-rich extracts is also possible, but matrix distortions have to be accounted for [21]. For such complex analytical problems, laser excited Shpol'skii spectrometry (LESS) is particularly useful.

Because of their relative polarity, PAH metabolites are not fully compatible with the matrix. However, the sensitivity can be greatly enhanced for phenolic hydroxy-metabolites if chemical derivatization (methylation) is applied. Utilizing enzymatic hydrolysis, derivatization, and LESS detection, monohydroxy metabolites of BaP can be determined at sub-ppb levels in the bile of fish.

Obviously, Shpol'skii spectrometry (and especially LESS) is not suitable as a rapid, low-cost, screening technique. It requires sophisticated, advanced instrumentation and cryogenic temperatures. An interesting result, however, is that the concentration of 3-OH-BaP in fish bile was found to be correlated with 1-OH-pyrene; the latter is easily determined with more conventional techniques, since its concentration is much higher. Synchronous fluorescence spectrometry (SFS) was found to be a very rapid and practical method; the sensitivity for this marker metabolite is sufficient for most field applications.

To date, contrary to the determination of PAHs, which is routinely carried out in numerous laboratories, the analysis of PAH metabolites has received minor attention. It should be realized that the amount of metabolites excreted is a direct measure for the integrated uptake, and that combining PAH metabolite levels with environmental levels of parent PAHs provides information on bioavailability. Evidently, the fluorescence techniques discussed in this chapter can play a significant role in this field of research.

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Chapter 14

Characterization of surfactants in water by desorption ionization methods

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14.1. INTRODUCTION

Surfactants represent one of the major and most versatile groups of organic compounds produced around the world. Their main uses are: industrial, 54% (cleaning products, food and industrial processing), household, 29% (laundry, dishwashing, etc.) and personal care, 17% (soaps, shampoos, cosmetics). The worldwide production in 1988 [1] was 2.8 million metric tons, with anionics, non-ionics and cationics representing 61%, 29% and 8%, respectively of the total surfactant production.

The nature of their uses, and the fact that most of the surfactants are water-soluble, results in their release into the aquatic environment. One of the main problems associated with the presence of surfactants is foaming in river and wastewater treatment plants. This

effect has been greatly reduced by the replacement of poorly biodegradable compounds, such as branched alkylbenzenesulfonates (ABS), by biodegradable linear alkylbenzenesulfonates (LAS). Other problems arise from the formation of toxic metabolites by biotransformation of some surfactants (i.e. polyethoxylated alkylphenols) under aerobic and anaerobic conditions [2,3], and from certain cationics which are toxic to microorganisms at high concentrations [4]. The presence of some surfactants or their by-products in the aquatic environment has been considered as a potential marker of pollution. Thus, the presence of ABS in groundwater has been used as an indicator of the groundwater's age [5]; linear alkylbenzenes can act as tracers of domestic wastes in the marine environment [6,7] and trialkylamines as indicators of urban sewage in sludges, coastal waters and sediments [8].

The analysis of surfactants in water is difficult because they are non-volatile compounds, highly polar and water-soluble. Common techniques for measuring surfactants are spectrophotometric and titrimetric methods, but they are non-specific and liable to interferences. For example, the methylene-blue method that is traditionally used as an indicator of potential anionic surfactant concentration is affected by the presence of other compounds with an anionic group and a hydrophobic moiety, and by organic cations and negative interferences [9]. It does not provide information on the individual surfactants present and has relatively high detection limits.

Chromatographic techniques such as gas chromatography (GC) allow the characterization of individual homologs but requires their prior derivatization to form more volatile derivatives and thus prevent their decomposition. Nevertheless, lower molecular weight members of each homologous series, and metabolites of non-ionic surfactants can be analyzed directly by GC. Liquid chromatography (LC) with fluorescence (FL) or UV detection is widely used for the analysis of anionics, although LC does not provide the high resolution of GC. Alkyl and ethoxylate chains of non-ionics of the alkylphenol type can be characterized by LC but this requires UV-absorbing or fluorescent derivatives to be used for polyethoxylated alcohols and ethylene/propylene oxide block copolymers. Conductivity or refractive index detectors are the most used for cationics. An excellent book on the analysis of surfactants by GC and LC techniques has recently appeared [10].

Mass spectrometry (MS) offers the advantages over the techniques cited above of providing the molecular weight distribution of surfactant oligomers and chemical information such as the degree of branching, the isomer distribution and the location of unsaturation and side chains. Because of their low volatility, surfactants cannot be measured by GC/MS. In order to make anionics amenable to GC/MS they are usually desulfonated, or derivatized to form sulfonyl chlorides [11], methylsulfonate esters [12] or trifluoromethane sulfonate derivatives [13]. In contrast, non-ionics (mainly alkylphenols) with a low degree of polyethoxylation ($n \leq 6$) have been analyzed by GC/MS, either intact or derivatized (i.e. as trimethylsilyl- or methyl esters for alkylphenol carboxylates) in commercial products [14] or environmental applications [15–22]. Trimethylsilyl derivatives of triethoxylated amines have also been reported [23].

LC/MS has been applied mainly to the analysis of non-ionic surfactants. LC is used either in normal or reversed phase mode, employing a moving belt [24] or thermospray interface [25,26]. Other reports deal with the use of an interface to permit LC/FAB/MS [27] or an ion spray LC/MS interface [28]. The peaks observed from an LC/MS system

equipped with a thermospray interface are mainly $[M + NH_4]^+$ with little fragmentation. Nevertheless, thermospray allows the differentiation of mixtures of non-ionics, even if they are not completely separated by the column. A characterization of a mixture of surfactants (anionic, non-ionic and cationic) by LC/MS, using thermospray, has been reported [29], and further environmental applications of this technique and LC/MS/MS have been published [30–33].

SFC/MS (supercritical fluid chromatography/MS) using CO_2 as mobile phase and chemical ionization has been used for the characterization of alcohol ethoxylates [34,35], whereas alkylphenol ethoxylates and alkyl diethanolamides have been analyzed using a mixture of ethanol and hexane as mobile phase [36]. Non-ionic surfactants have also been characterized by SFC/MS [37] and SFC/MS/MS [38].

The use of desorption ionization techniques such as field desorption (FD), fast atom bombardment (FAB), desorption chemical ionization (DCI), laser desorption (LD) and, more recently, electrospray (ESI) has grown considerably over the last few years, due to their ability to ionize complex organic molecules which are not amenable to more conventional methods such as electron impact (EI) or chemical ionization (CI). The combination of these techniques with tandem mass spectrometry (MS/MS) can be used to separate individual components in a mixture, or to obtain structural information on a single component. The reader is referred to many excellent articles which have been published on the theory and applications of soft ionization methods [39–44].

This chapter is devoted to the characterization of surfactants and their identification in the aquatic environment by these desorption ionization mass spectrometric techniques.

14.2. CHARACTERIZATION OF SURFACTANTS

14.2.1. Anionics

14.2.1.1. Fast atom bombardment

The mass spectra of alkyl sulfates and sulfonates, alkylbenzenesulfonates, alcohol sulfates, alcohol ether sulfates and phosphates, α -olefin sulfonates, fatty acid salts, sulfosuccinate diesters and *N*-acetyl amino acids have been studied [45–48]. Samples were analyzed neat or dissolved in a glycerol, thioglycerol or triethanolamine matrix.

The spectra of alkylsulfates ($C_nH_{2n+1}OSO_3Na$), alkylsulfonates ($C_nH_{2n+1}SO_3Na$) and alkylbenzenesulfonates ($C_nH_{2n+1}-C_6H_4-SO_3Na$) exhibited a series of cationized molecular ions of the formula $[mM + Na]^+$ (for $m = 1-7$) in the FAB positive mode (see Fig. 14.1) and $[M - Na]^-$ and $[2M - Na]^-$ in the negative mode.

The FAB(+) of alkylbenzenesulfonates also showed peaks at two mass units below the most intense $[M + Na]^+$ ions ($m/z = 343, 357, 371, 385, 399$ for $C_{10}-C_{14}$ homologs) that were identified by Field et al. [49] as dialkyltetralinsulfonates (DATS), which are reaction by-products in the LAS synthesis.

Alkylphosphates $C_nH_{2n+1}OPO_3HX$ ($X = Na, K$), in the positive FAB mode, show intense peaks corresponding to mono- and diesters and less intense ions from pyroester, as well as different fragment ions. The profile of the spectra is greatly influenced by the per-

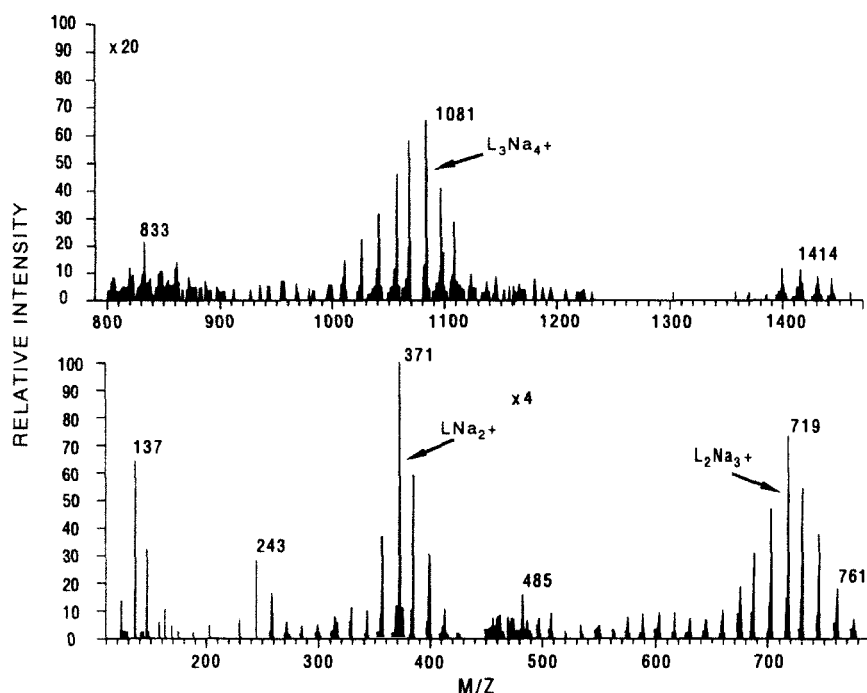


Fig. 14.1. FAB(+) spectrum of alkylbenzenesulfonates. L is the anion. Reproduced with permission from [46] © 1984 by American Chemical Society).

centages of di- and pyroester. Thus, Fig. 14.2 [50] shows the FAB(+) spectrum of monosodium laurylphosphate from two different manufacturers, using triethanolamine as matrix. The first compound shows as base peak the $[M' + Na]^+$ ($m/z = 479$) and $[M' + H]^+$ ($m/z = 457$) ions of the diester (M'). The monoester (M) shows mainly $[M + Na]^+$ and $[M + 2Na]^+$ ($m/z = 311$ and 333 , respectively) and weak $[M + H]^+$ ($m/z = 289$). Clusters of $[2M' + Na]^+$ type for the diester are also observed. On the other hand, the second compound shows the monoester as base peak, with intense $[M + H]^+$, $[M + Na]^+$ and $[M + 2Na]^+$ ions. Clusters of $[2M + Na]^+$ type at $m/z = 599$ from the monoester are also observed. Both compounds showed no ions related to the presence of pyroester. The other ions of the spectrum arise from unreacted phosphoric acid. Thus, $m/z = 125$ probably corresponds to $[H_2PO_3 + 2Na]^+$, $m/z = 142$ to $[H_3PO_4 + 2Na]^+$ and $m/z = 167$ to $[H_3PO_4 + 3Na]^+$.

The negative mode, which is especially suitable for anionics, also showed intense peaks at $m/z = 80$ $[SO_3]^-$ for sulfonates and sulfosuccinates, at $m/z = 80$ for $[SO_3]^-$, at 96 $[SO_4]^-$ and 97 $[HSO_4]^-$ for sulfates and $m/z = 79$ $[PO_3]^-$ for phosphates.

Structural identification can be achieved by collision activated dissociation (CAD) of selected ions. Figure 14.3 shows the product-ion mass spectrum from the collisionally activated dissociation (CAD) of the molecular anion of C_{12} ABS at $m/z = 325$ (left), and the same, but from the molecular anion of C_{12} LAS also at $m/z = 325$ (right) [51]. The product ion of highest abundance from the molecular anion of ABS ($m/z = 197$) corresponded

to a propylene-substituted benzenesulfonate structure [46]. The most abundant product-ion from the molecular anion of LAS ($m/z = 183$) is an ethylene-substituted benzenesulfonate ion. The other ions present ($m/z = 133$ for ABS and $m/z = 119$ for LAS) are propylene- and ethylene-substituted phenoxide ions, according to Scheme 14.1 proposed by Borgerding and Hites [51]. The use of a quadrupole (low energy CAD process) or a magnetic sector instrument (high energy CAD) can affect the product-ion spectrum. Thus, no signal from benzenesulfonate ions having alkyl chain lengths between the parent ion and the most abundant product ion was observed using a quadrupole [51]. Product ions at 14-Da intervals, corresponding to benzenesulfonates with alkyl substituents that are inter-

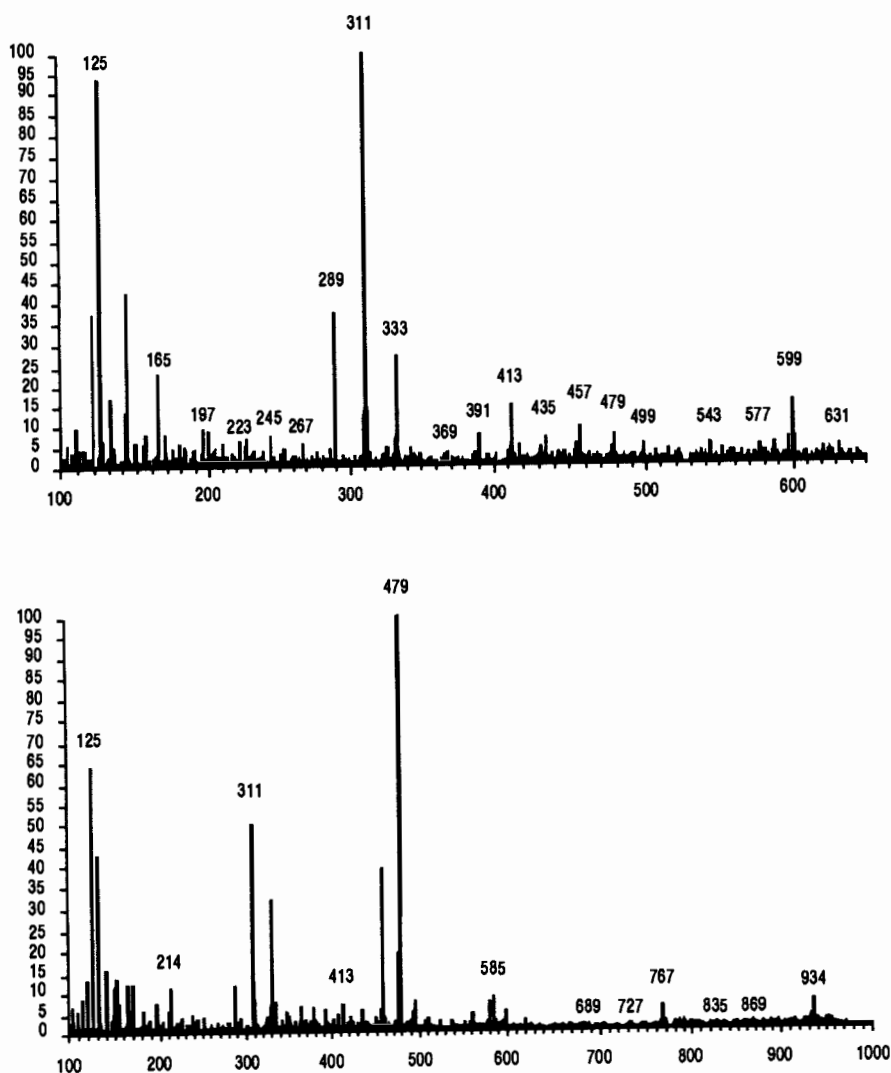


Fig. 14.2. FAB(+) spectra of monosodium laurylphosphate from two different manufacturers.

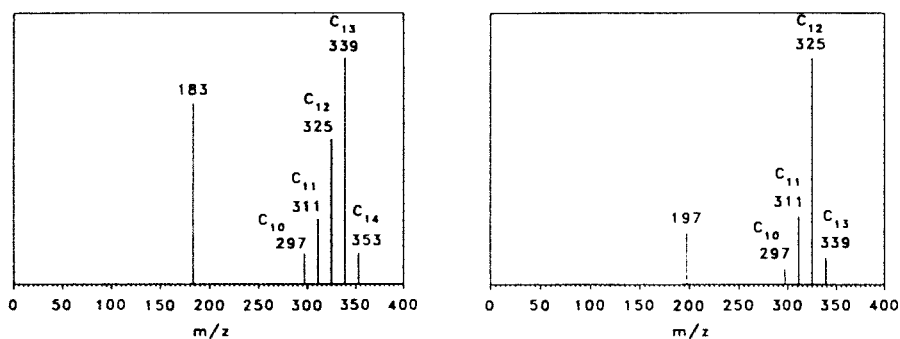
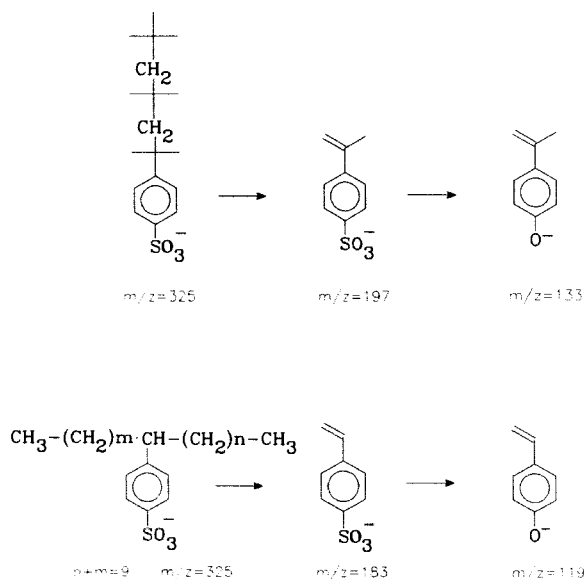


Fig. 14.3. (Left) Product ion spectrum from the CAD of the molecular anion of C_{12} branched alkylbenzenesulfonate (ABS) at m/z 325. (Right) Same but from the molecular anion of C_{12} linear alkylbenzenesulfonate (LAS) also at m/z 325. Reproduced with permission from [51] © 1992 by American Chemical Society).

mediate between the parent ion and the most abundant product ion, were present when a magnetic instrument was used [46]. These ions were thought to arise by losses of C_nH_{2n+2} from decompositions remote from the charge center [52]. They are analogous to those described first for long chain fatty acid anions [53], with a proposed mechanism involving a 1,4-loss of H_2 to give a terminally unsaturated anion and a 1-alkene.

Alkylphenyl ether sulfates, sulfonates and phosphates [$C_nH_{2n+1}-C_6H_4-(OCH_2CH_2)_mX$] ($X = OSO_3Na$, SO_3Na or OPO_3H_2 , respectively) in the negative FAB mode give



Scheme 14.1.

$[R(EO)_mSO_3]^-$ ions, where m is the number of ethylene oxide (EO) units. Adducts of the $[2M + Na - 2H]^-$ type are also observed [48]. Identification of the alkylphenyl part of the surfactant can be rationalized via phenoxide ions $[C_nH_{2n+1}-C_6H_4-O]^-$ [47].

The CAD spectra of negative ions from alkylphenyl ethers show fragments at 14-Da intervals, indicative of the aliphatic structure until the beginning of the EO units; this is

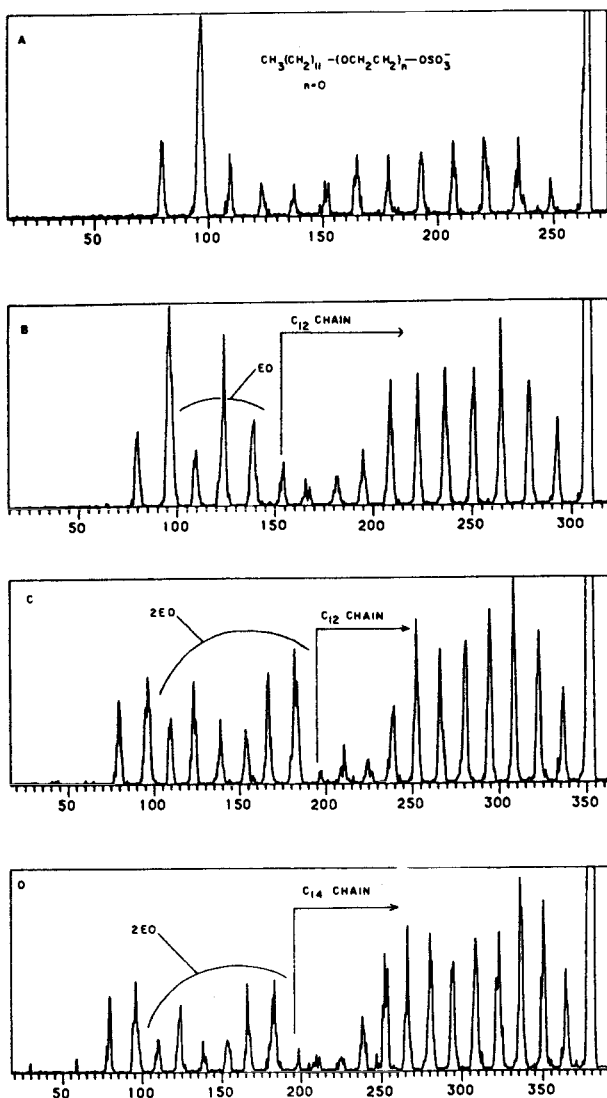


Fig. 14.4. CAD spectra of negative ions from ammonium lauryl ether sulfate: (a) m/z 265, the molecular anion containing no EO units; (b) m/z 309, the ligand with one EO; (c) m/z 353, the ligand containing two EO units; and (d) m/z 381, the tetradecyl homolog containing two EO units. Reproduced with permission from [46] © 1984 by American Chemical Society.

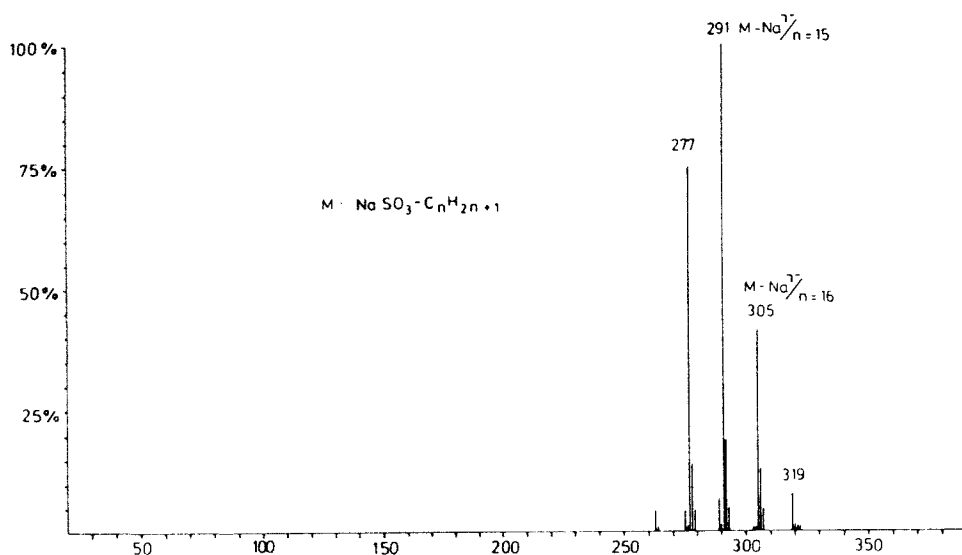


Fig. 14.5. Negative FD spectrum of a linear alkylsulfonate. Reproduced with permission from [45] © 1983 by Springer-Verlag.

preceded by a group of low intensity peaks which indicates the end of the alkyl chain. The length of the alkyl chain can be determined by counting the peaks between the precursor ion and the EO units. They can be deduced by counting the number of peaks differing by 44-Da, starting from $m/z = 80, 96$ or 79 (sulfonates, sulfates or phosphates) upwards. Again, all ions resulting from losses of C_nH_{2n+2} (14-Da intervals) are formed from decomposition remote from the charge center.

Alkyl ether sulfates $[C_nH_{2n+1}-(OCH_2CH_2)_m-OSO_3Na]$ exhibit a FAB behavior similar to those described above for alkylphenyl ether sulfates. Similarly, the FAB-CAD spectra of alkyl ether sulfates (Fig. 14.4) give the C_nH_{2n+2} losses as well as the oxygen-containing fragment. All ions resulting from these losses are observed for branched sulfonates and alkyl ether sulfates, but the relative abundances of the ions in the envelope of the appearance or loss pattern are perturbed with respect to the abundance observed in the unbranched alkylsulfates [52].

Sodium and potassium salts of fatty acids of the general formula $C_nH_{2n+1}COONa$ (C_{12} – C_{18} alkyl chain), which are used as soaps, yield poor responses in the positive ion mode. The negative ion spectra, however, produce intense carboxylate anion signals but also matrix adducts.

14.2.1.2. Field desorption

Alkylsulfonates and alkylbenzenesulfonates are the main anionic surfactants which have been studied [45,54–60]. Their positive FD spectra are dominated by $[M + Na]^+$ ions and clusters of the $[nM + Na]^+$ type, although both M^+ and $[M + H]^+$ ions are also present [55]. By using negative ion FD, the formation of $[M - Na]^-$ ions is observed

[45,57,59] (see Fig. 14.5). An additional advantage of negative FD is that mixtures of surfactants do not need to be separated prior to analysis because neither non-ionics nor cationics are sensed. The emitter current plays an important role in FD spectra. Thus, Shiraishi et al. [56] found that $[2M + Na]^+$ ions have a relatively high intensity at a lower emitter current, but at higher values the intensity of this cluster decreases rapidly whereas the $[M]^+$ and $[M + Na]^+$ ions increase. As FD does not give fragment ions, it is usually combined with CAD-MS/MS experiments in order to provide structural information.

The FD-CAD spectra of alkylsulfonates, that is of $[M + Na]^+$, the parent ion, have been studied [58]. The main intense fragments, apart from sodium, give peaks at $m/z = 62$ and 126, corresponding to $[Na_2O]^+$ and $[Na_2SO_3]^+$, respectively. This last fragment, formed by loss of the alkyl substituent, allows rapid determination of the length of the alkyl chain. All the studied alkylsulfonates presented an intense fragment at $m/z = 154$ that is assigned to branching of the alkyl chain at the carbon α to the sulfonate moiety $[Na_2SO_3CHCH_3]^+$.

The FD-CAD spectrum of alkylbenzenesulfonates [58,59] again shows the fragments at $m/z = 23$, 62 and 126. The most intense ion in the upper mass range ($m/z = 230$) with the structure $[Na_2SO_3-C_6H_4-CHCH_3]^+$, arises from benzylic cleavage of the alkyl chain at the same branching position found for alkylsulfonates. Loss of the entire alkyl chain leads to the ion $[Na_2SO_3C_6H_4]^+$ at $m/z = 202$, with a lower abundance than that at $m/z = 230$. A series of ions with the assigned structure $[Na_2SO_3-C_6H_4-C_nH_{2n}]^+$ is observed between the precursor ion and the fragment at $m/z = 230$, which further facilitates the determination of the alkyl chain. Finally, the presence of the fragment at $m/z = 91$ $[C_7H_7]^+$ is indicative of an aromatic substituent.

14.2.1.3. Other desorption ionization methods

Schneider et al. [45] reported the DCI spectra of alkylsulfonates. These exhibit abundant fragmentation but little structural information. The $[M + Na]^+$ ions were more intense than $[M + H]^+$. The relative abundance of $[M + Na]^+$ ions depended on the DCI probe, being more abundant when the probe tip was coated with a polyimide. However, good spectra for alkylbenzenesulfonates could not be obtained. Laser desorption-Fourier transform mass spectrometry (LD/FTMS) has been used to characterize alkyl sulfates and linear alkylbenzenesulfonates (LAS) on textiles [61]. The LD spectra of alkyl sulfates give only $[M - Na]^-$ and $[2M - Na]^-$ ions, the latter being the more abundant. On the other hand, a low abundance for dimers was observed for LAS. The types of ions observed on laser desorption were essentially the same, and independent of the wavelength, laser energy or sample state. As LD favors the more surface-active molecules, the quantification of homolog mixtures (i.e. LAS) requires the use of internal standards, preferably the deuterated analog of each homolog. In earlier laser ionization studies of sodium alkyl sulfates and sulfonates [62,63], the spectra showed $[M + Na]^+$ ions together with lower $[2M + Na]^+$ ions and inorganic ions such as $[Na_2SO_4]^+$, but no fragmentation was observed. LD/FTMS and FAB have been applied to the analysis of cationic and anionic surfactants [64]. Some advantages of LD/FTMS include the elimination of the liquid matrix, laser desorption/post-ionization to provide library-searchable EI spectra of surfactants, the high resolution measurement of the parent ions, the accurate measurement of parent

and product ions, and high sensitivity. The anionics, which were characterized, $[\text{CF}_3-(\text{CF}_2)_{11}-\text{SO}_3\text{K}_2]$ by these two methods showed nearly identical spectra, although FAB presented abundant low mass matrix ions, whilst the LD spectrum showed essentially the molecular cation.

Anionic surfactants (LAS) have also been studied by negative-ion plasma desorption mass spectrometry (PD/MS) with Nytran, a quaternized Nylon-66, being used as a support [65]. The spectrum showed an abundant molecular anion and the known fragment ion at $m/z = 183$, although no response was obtained in the positive mode. Anionics exhibit abundant $[\text{M} + \text{Na}]^+$ ions in positive PD, with nitrocellulose as a support. Recently, the characterization of anionic surfactants by positive and negative ion electrospray ioniza-

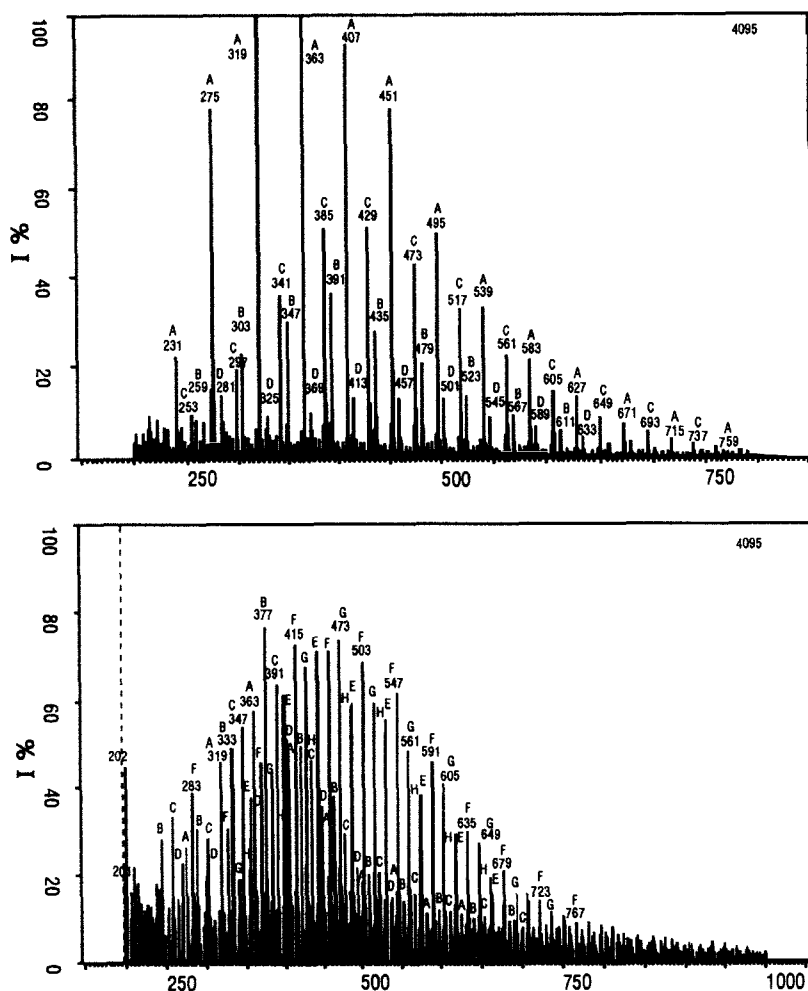


Fig. 14.6. FAB(+) spectrum of polyethoxylated fatty alcohols. (Top) from natural source. (Bottom) from synthetic source. Reproduced with permission from [48] © 1989 by John Wiley & Sons Ltd.

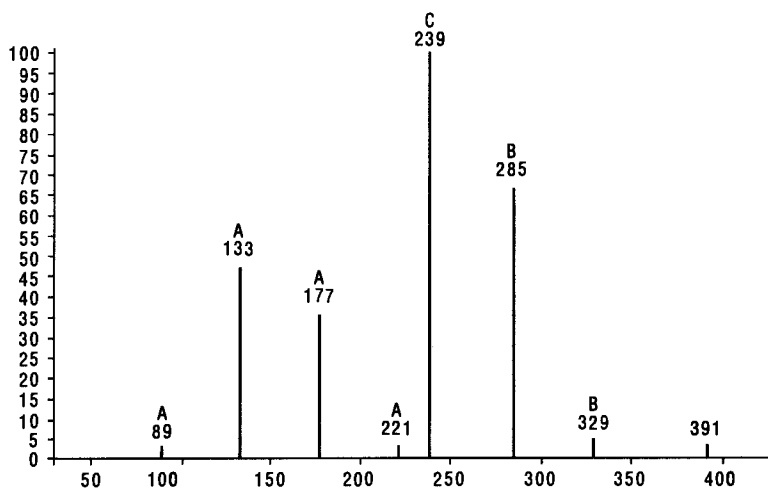


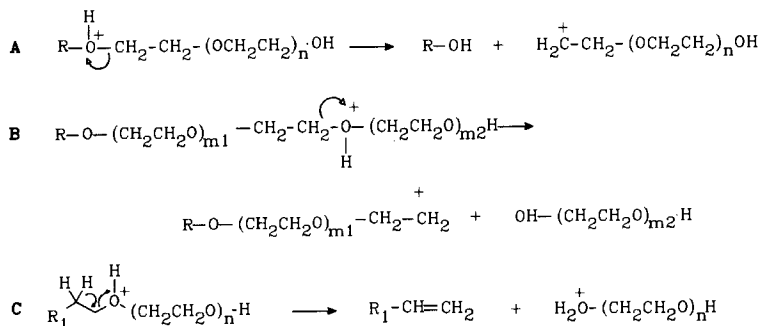
Fig. 14.7. Product ion spectrum of pentaethoxylated myristyl alcohol. Parent ion $[M + H]^+ = 435$. Reproduced with permission from [70] © 1991 by American Chemical Society.

tion/mass spectrometry (ESI) have been reported [66]. The anionics showed $[M - Na]^-$ ions in the negative mode and $[M + Na]^+$ and $[M + H]^+$ ions in the positive mode. The ESI conditions were as follows: needle voltage (-3 to -4 kV) and ($+2$ to -3 kV) for positive and negative ions, respectively. The bath gas and temperature were ($N_2/200$) and ($O_2/150-200$), the solvent was alcohol/water (typically 50:50 methanol/water or 75:25 isopropanol/water) at flow rates of $0.5-3 \mu\text{l/min}$.

14.2.2. Non-ionics

14.2.2.1. Fast atom bombardment

Non-ionics are the second most used group of surfactants. Polyethoxylated alkylphenols, alcohols, fatty acids and amines have been characterized [47,48,67,68]. Intense



Scheme 14.2.

FAB(+) spectra can be obtained without a matrix [68], or using thioglycerol [48] or thioglycerol saturated with NaCl [67]. Non-ionics have also been used as internal standards for exact mass measurements in FAB [69,70].

FAB spectra of non-ionics are particularly suitable in the positive mode. They are dominated by protonated and/or cationized molecular ions, $[M + X]^+$ ($X = H, Na$ or K) separated by 44 Da corresponding to different degrees of polyethoxylation.

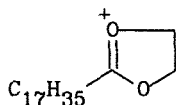
Figure 14.6 shows how positive FAB can differentiate the natural or synthetic source [48] of polyethoxylated alcohols $[C_nH_{2n+1}O(CH_2CH_2O)_mH]$. The apparently complex spectrum of alcohols from natural sources (Fig. 14.6, top) is formed mainly by $[M + H]^+$ ions (A and B type for C_{12} and C_{14} alcohols, respectively) and $[M + Na]^+$ ions (C and D type for both C_{12} and C_{14}). The FAB(+) spectrum of polyethoxylated alcohols from a synthetic source presents a more complex pattern (Fig. 14.6, bottom) and is formed by all possible combinations of $[M + H]^+$ and $[M + Na]^+$ ions of C_{12} – C_{15} alcohols. In the negative FAB mode, matrix ions dominate the spectrum, even with high concentrations of the surfactants in the matrix.

MS/MS of polyethoxylated fatty alcohol $[M + H]^+$ ions has been studied [38,70]. The product-ions observed follow the same mechanism as described for polyethylene glycols (PEG) [71–73]. Figure 14.7 shows as an example the CAD mass spectrum of pentaethoxylated myristyl alcohol [70]. The product-ions can be rationalized according to Scheme 14.2 [38,70]. Thus, A- and B-type ions are formed by charge site-initiated decomposition, whereas the C-type are formed by ether cleavage and hydrogen transfer that leads to protonated PEG. This last fragmentation can be explained under high CAD conditions [74] but is unexpected under low CAD conditions [38].

The CAD spectra of secondary and branched alcohol ethoxylates do not show B-type ions [38] and therefore it is possible to distinguish linear, primary alcohol ethoxylates from the other alcohol ethoxylates. However, although alkene loss aids one in determining the hydrophobe, the hydrophilic chains and A series fragment ions dominate the CAD spectra. No structural information can be inferred regarding the hydrophobe for secondary or branched alcohol ethoxylates.

The FAB(+) spectra of polyethoxylated alkylphenols $[C_nH_{2n+1}-C_6H_4-O-(CH_2CH_2O)_mH]$ show weak $[M + H]^+$ ions or strong $[M + Na]^+$ ions when salt is added, corresponding to different degrees of polyethoxylation. The lower part of the spectrum shows benzylic cleavage of the alkyl chain by a series of ions at $m/z = 135, 179, 223, 267, \dots$ in agreement with those observed in its EI spectrum [75]. In the negative mode, an intense base peak is observed, corresponding to a phenoxide ion of the same structure as described for alkylphenyl ether sulfates [47].

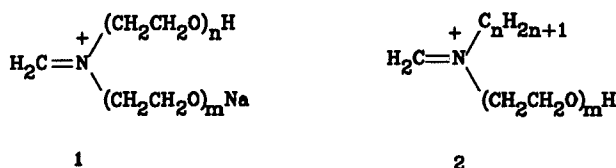
Figure 14.8 shows the FAB(+) spectrum of a commercial polyethoxylated fatty acid [67]. The example corresponds to stearic acid with 9 mol of ethylene oxide. The base peak at $m/z = 311$ corresponds to



whereas different $[M + Na]^+$ ions up to $n = 12$ can be identified. The spectrum range $m/z = 400$ – 800 shows impurities of polyethoxylated palmitic and oleic acids, whereas at

800–1200 these compounds are masked by impurities of C_{12} – C_{16} alcohols. The presence of minor amounts of other acids seems to be due to tall oil in the raw material.

Polyethoxylated fatty amines $(C_nH_{2n+1})N[(CH_2CH_2O)_xH][(CH_2CH_2O)_yH]$ are usually derived from coconuts or beef tallow. Their FAB(+) spectra [67,68] reflect an important background which depends on the nature of the sample. Thus, the FAB(+) spectrum of pentaethoxylated tallow amine exhibits $[M + Na]^+$ and $[M + H]^+$ ions corresponding to the different fatty acids of beef tallow (C_{14} , C_{16} , C_{18} , C_{18-} and $C_{18=}$) and different degrees of polyethoxylation. Intense series of fragment ions resulting from loss of methanol from the molecule [47,68] are observed. Other informative fragments are formed by α -cleavage of an ethoxylate chain 1 (i.e. $n + m = 2-4$ gives $m/z = 140$, 184 and 228) or an alkyl chain 2 (i.e. for $n' = 1-3$ one sees $m/z = 298$, 342 and 386, respectively).



The spectrum of polyethoxylated coconut fatty amine displays the composition of coconut oil (C_8 – C_{18} even numbered fatty acids, the main one being the C_{12} acid). The strongest peaks correspond to $[M + Na]^+$ ions with different degrees of polyethoxylation, and fatty acids. The types of fragments described for tallow amines are present.

Ethylene oxide (EO)/propylene oxide (PO) block copolymers, the most commonly used surfactants of this type, are compounds derived from nonylphenols or alcohols in the C_9 – C_{11} range. Figure 14.9 shows, as an example, the positive FAB spectrum of a commercial nonylphenol with n mol of EO and m mol of PO, where $n = 8.6$ and $m = 5$, according to the manufacturer's information. The spectrum is very complex, giving all the possible combinations from nonylphenol and EO and PO. The main peaks arise from $[M + Na]^+$ ions (thioglycerol + NaCl was used as matrix) in different combinations. Thus, in this particular case, the main series is $(n,3)$ where $n = 10$, and $[M + Na]^+ = 681$ for $(6,3)$. Other series ranging from $(n,1)$ to $(n,5)$ are easily observed, with $[M + Na]^+ = 521$,

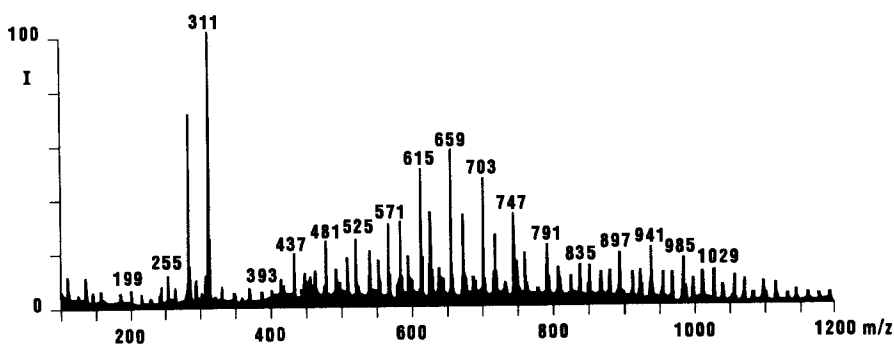


Fig. 14.8. FAB(+) spectrum of polyethoxylated stearic acid with $(n = 9)$. Reproduced with permission from [67] © 1989 by Pergamon Press plc.

667, 695 and 797 for (5,1), (7,2), (5,4) and (6,5), respectively. The presence of free ethylene-propylene block copolymers is observed in the lower part of the spectrum. Thus, the series of sodiated ions (n,m) with $m = 1-4$ and $n \leq 10$ is apparent. For example, $m/z = 183$, 227 and 271 correspond to ($n,2$) etc.

The FAB spectra of block copolymers can be more complex if the raw materials are alcohols. Thus, all combinations of different alcohols and EO and PO are observed, leading to numerous $[M + Na]^+$ ions formed by overlapping of two or more compounds. MS/MS allows the unequivocal differentiation of the overlapped compounds and it is useful to know how the ethylene and propylene oxide units are linked.

Another group of non-ionic surfactants that has been extensively studied [47,48,67] is the polyethoxylated alkanolamides $C_nH_{2n+1}-CO-N[(CH_2CH_2O)_xH][(CH_2CH_2O)_y)H]$. The most commonly used are coconut- and lauric-mono- and diethanolamides. Figure 14.10 displays the positive and negative FAB spectra of a commercial coconut diethanolamide [48], using thioglycerol as matrix. The spectrum in its positive mode (Fig. 14.10, top) exhibits intense $[M + H]^+$ ions (A series) and $[M + Na]^+$ ions (B series) corresponding to diethanolamide derivatives of C_8 ($m/z = 232$, 254), C_{10} ($m/z = 260$, 282), C_{12} ($m/z = 288$, 310), C_{14} ($m/z = 316$, 338), C_{16} ($m/z = 344$, 366), C_{18} ($m/z = 370$, 392) and C_{18} ($m/z = 372$, 394). The base peak at $m/z = 106$ $[HN-(CH_2CH_2OH)_2 + H]^+$ indicates the presence of unreacted diethanolamine, as determined by MS/MS (parent ion mode) [48]. Monoethanolamines show a similar ion at $m/z = 62$ as the base peak, corresponding to $[HN-(CH_2CH_2O) + H]^+$. The relative intensity of ions can be enhanced by addition of NaOH or NaCl to the matrix, resulting in the formation of $[M + Na]^+$ ions which dominate the spectrum. In the negative mode (Fig. 14.10, bottom), the carboxylate anions of coconut fatty acids give the most intense ions. They might arise from the unreacted esters, or fatty acid impurities. Nevertheless, Righton and Watts [47] observed intense $[M - H]^-$

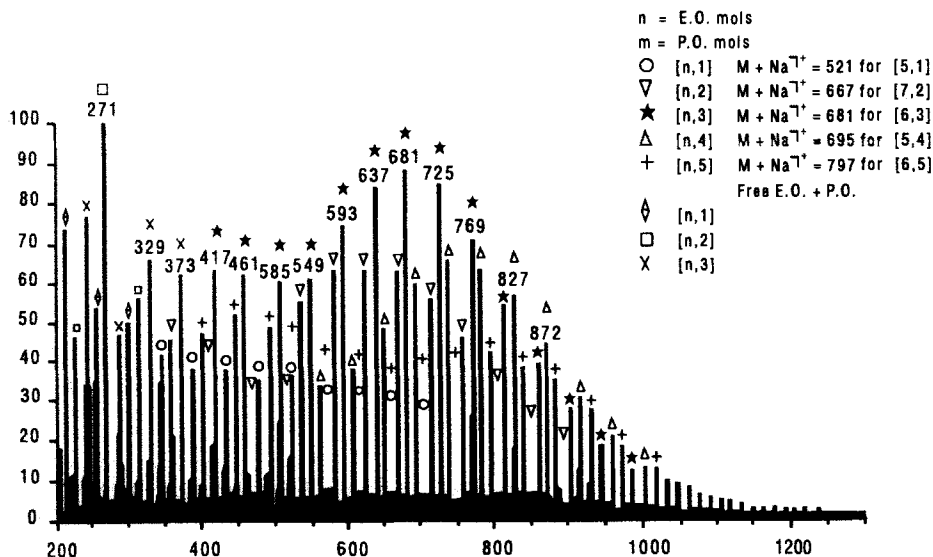


Fig. 14.9. FAB(+) spectrum of a polyethoxypropoxy nonylphenol block copolymer.

ions together with ions resulting from loss of $\text{CH}_2\text{CH}_2\text{OH}$ groups from the molecule, when an alkylmonoethanolamide mixture was studied.

Alkylimidazolines $\text{C}_n\text{H}_{2n+1}-\text{C}_3\text{H}_4\text{N}-(\text{CH}_2\text{CH}_2\text{O})_m\text{H}$, give intense $[\text{M} + \text{H}]^+$ ions corresponding to the different alkyl homologs present. These compounds exhibit fragment ions

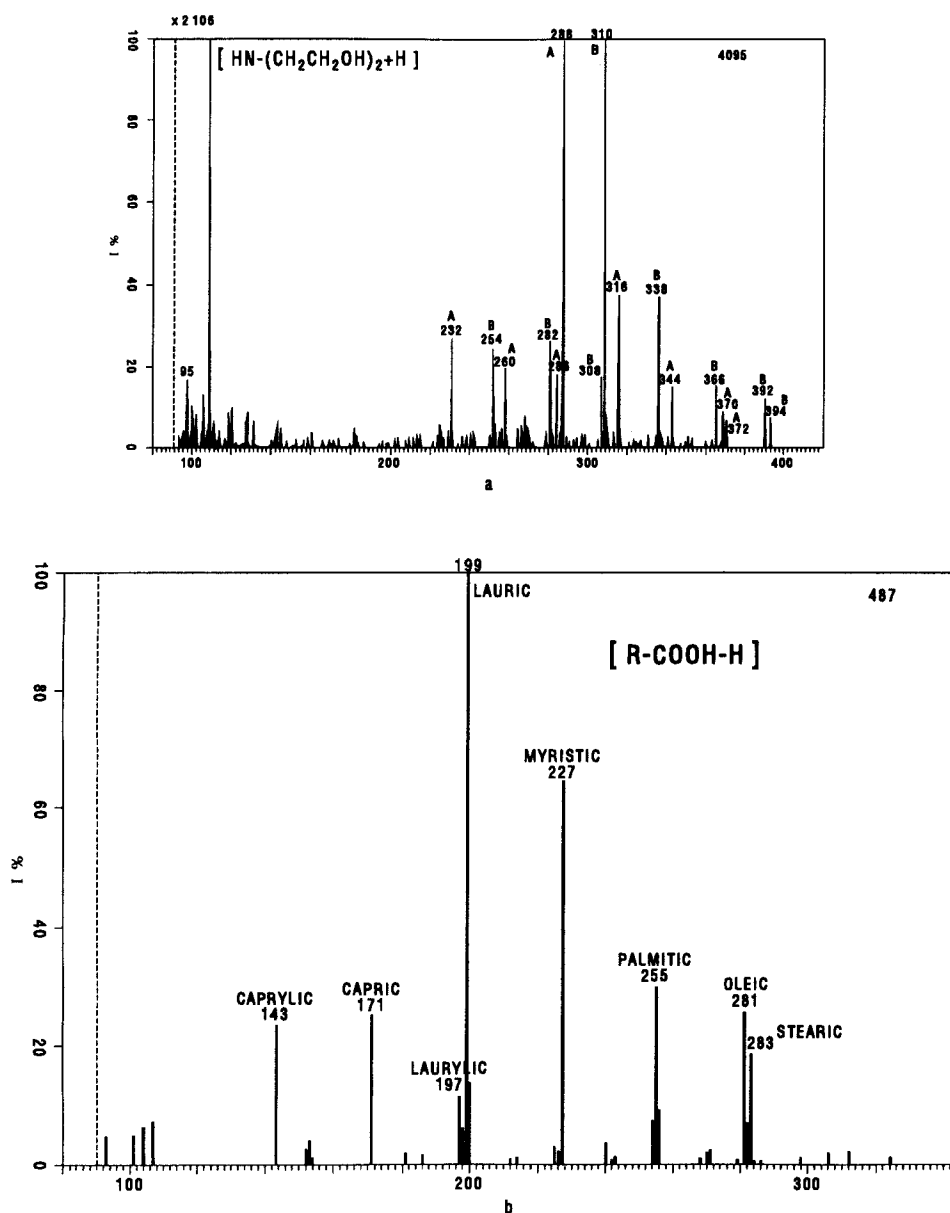


Fig. 14.10. (Top) FAB(+) and (bottom) FAB(-) spectra of commercial coconut diethanolamide. Reproduced with permission from [48] © 1989 by John Wiley & Sons Ltd.

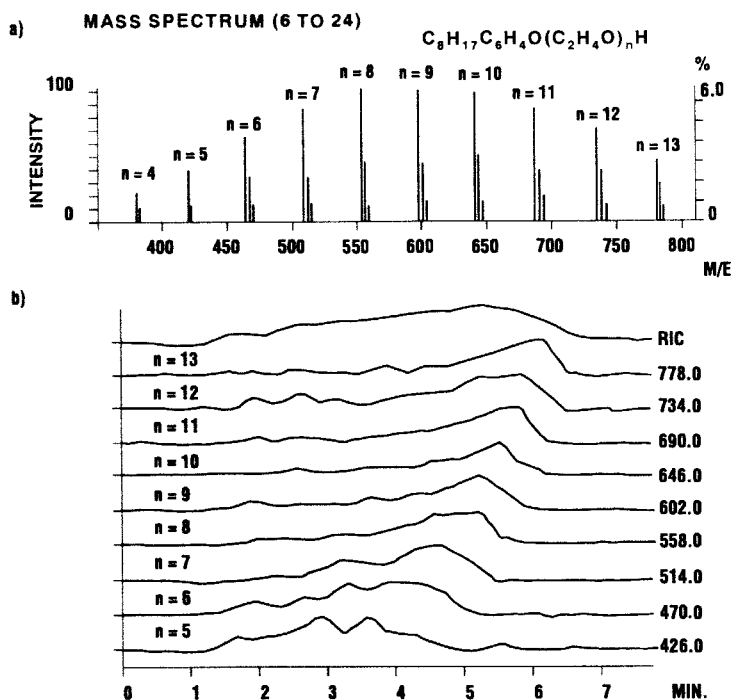


Fig. 14.11. (a) FD spectrum of polyethoxylated octylphenol; sum of the stored spectra, small peaks are neglected. (b) Reconstructed mass chromatogram; current programming rate 3 mA/min. Reproduced with permission from [56] © 1979 by The Chemical Society of Japan.

apparently due to loss of the substituent group on N-3 of the imizadoline ring with concurrent transfer of a hydrogen atom [47].

Amine oxides $C_nH_{2n+1}-NO(CH_3)_2$ exhibit intense peaks for $[M + H]^+$ and proton bound dimers $[2M + H]^+$, as well as other ions resulting from loss of water [47,48,76]. No $[M + Na]^+$ ions were observed even though salt was added to the matrix. In the negative mode some authors [47] found intense fragment and $[M - H]^-$ ions, in contrast to other reported unsuccessful results [67,76].

Lyon et al. [67] studied the behavior of dimethyldicocoamine oxide by MS/MS. The CAD spectrum of the $[M + H]^+$ ($m/z = 230$) shows an intense fragment owing to loss of water ($m/z = 212$). Nevertheless, the most abundant fragment ion is at $m/z = 58$, coming probably from cleavage of the long alkyl chain β to the nitrogen, with the concomitant loss of the $-OH$ from the parent ion. Finally, the peak at $m/z = 61$ is formed by direct cleavage of the C-N bond of the lauryl moiety with the oxygen remaining attached to the nitrogen.

14.2.2.2. Field desorption

The main non-ionic surfactants studied by FD and FD-CAD are polyethoxylated alkylphenols, amines and Spans® [56,58,60,68].

Polyethoxylated alkylphenols present a series of intense $[M]^+$ [56] or $[M + H]^+$ [68] or $[M + H + Na]^+$ [60] ions spaced by 44 Da from different EO units. In contrast with the FAB results, no fragmentation is observed under FD conditions. The distribution pattern is also dependent upon the emitter current [56]. At low values, the distribution pattern of $[M]^+$ ions is most intense for low oligomers, whereas at higher emission currents the intensity is displaced to higher oligomers (see Fig. 14.11), suggesting that a fractional desorption is caused by the thermal gradient [56].

The FD-CAD spectra of polyethoxylated alkylphenols allow one to obtain structural information not provided by FD spectra. Thus, Weber et al. [58] found that the main product ions from the parent $[M + H]^+$ ion were $[(CH_3)_2C-C_6H_4-(OCH_2CH_2)_mH]^+$, which indicates how the alkyl chain is branched, and a series of fragments due to cleavage of the unbranched alkyl chain $[C_nH_{2n}C(CH_3)_2-C_6H_4-(OCH_2CH_2)_mH]^+$. These last fragments are not observed under EI conditions. An intense fragment from cleavage of the ether bond and branched alkyl chain $[(CH_3)_2C-C_6H_4-OCH=CH_2]^+$ is observed, together with the homologous series from cleavage of the unbranched alkyl chain, and the series with some EO units $[C_nH_{2n}C(CH_3)_2-C_6H_4-(OCH_2CH_2)_mOCH=CH_2]^+$. Rearrangement and cleavage of the alkyl chain leads to alkylphenol ions $[C_nH_{2n}C_6H_4OH]^+$. Other intense ions formed by ether cleavage $[(CH_2CH_2O)_mH]^+$ characterize the hydrophilic substituent.

The main product ions observed in the FD-CAD spectra of polyethoxylated fatty amines [58], derived from $[M + H]^+$ as parent ion, are $[(CH_2CH_2O)_mH]^+$, and those formed by α -cleavage of the alkyl chain or the EO chain, $[H_2C=N^+((CH_2CH_2O)_n-H)(CH_2CH_2O)_mH]$ and $[C_nH_{2n}-N^+(=CH_2)(CH_2CH_2O)_mH]$, respectively. Cleavage of the ether bond, and loss of the alkyl chain as olefin, hydrocarbon and ammonium ions are also present with low intensity.

The FD spectrum of Span® compounds, which are partial esters of the common fatty acids (C_{12} – C_{18}) and hexitol anhydrides (hexitans and hexides) derived from sorbitol, exhibit $[M + H]^+$ ions of mono-, di- and triesters of the sorbitan, the mono- and diester of sorbid, as well as fragment ions $[RCO]^+$ of unreacted fatty acids [56].

14.2.2.3. Other desorption ionization methods

Nonylphenol ethoxylates have been analyzed by DCI [68]. The authors reported that in contrast to the FAB technique, the DCI leads to the generation of abundant $[M + H]^+$ ions even for surfactants with a long ethoxylate chain. Nevertheless, the DCI spectrum does not give any information on the presence of the alkylphenol group. Laser desorption Fourier transform ion cyclotron resonance mass spectrometry LD/FT/ICR/MS has been used to determine the molecular weight distributions (up to 3500 Da) of octylphenol ethoxylates (see Fig. 14.12) [77]. As laser desorption-ionization produces minimal fragmentation, it allows an accurate measurement of the relative abundance of neutral oligomers without the need for prior chromatographic separation of the components. The presence of poly(ethylene oxide) side products of the polymerization process, which are not observed by LC/UV has also been determined by LD/FT/ICR/MS. Ethoxylated fatty amines were analyzed by DCI [68]. The spectrum (see Fig. 14.13) shows several series of $[M + H]^+$ ions corresponding to different degrees of ethoxylation and the number of carbon atoms in the alkyl chain. In addition, structure specific fragments formed by α -cleav-

age of one ethoxylate chain $[C_nH_{2n+1}-N^+(=CH_2)(CH_2CH_2O)_mH]$ ($m = 1,2$) are observed. Abundant $[M + H]^+$ ions for non-ionic surfactants are obtained by using PD in the positive mode and nitrocellulose as a support [65]. Non-ionic surfactants analyzed by ESI with the operating conditions described before [66], gave $[M + Na]^+$ ions in the positive mode, with unsuccessful results in the negative mode.

14.2.3. Cationics

14.2.3.1. Fast atom bombardment

Aliphatic amines, amine salts, fatty quaternary ammonium salts, benzylalkyldimethyl ammonium salts and ethoxylated quaternary amines are the major classes of cationic surfactants studied [47,48,67,68,76,78]. They can be analyzed neat or dissolved in a glycerol, thioglycerol or triethanolamine matrix.

The general behavior of cationics under FAB(+) is to show the presence only of molecular ions, with little fragmentation or impurities arising from unreacted material [48]. In the negative mode, FAB spectra can be used to identify the anionic counterion.

Aliphatic amines $C_nH_{2n+1}NH_2$ are chemical intermediates in the production of many cationics. The FAB(+) spectrum shows the corresponding $[M + H]^+$ ions and relatively intense ions 2 Da lower; these arise from loss of a hydrogen molecule from the parent ion, as confirmed by MS/MS [76]. Other structural information from the CAD spectrum is given by the ammonium ion ($m/z = 18$) and an alkyliminium ion at $m/z = 30$ $[CH_2NH_2]^+$ characteristic of a primary amine. Lyon et al. [76] suggested that this fragment probably

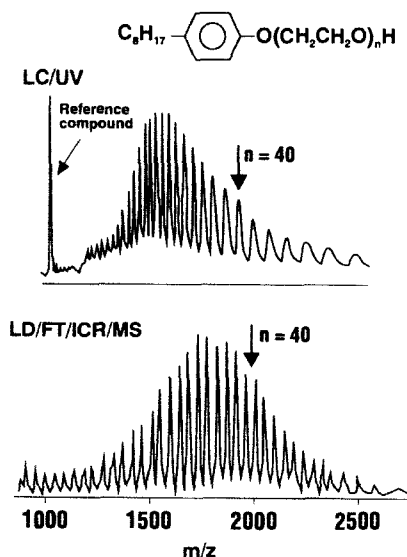


Fig. 14.12. (Top) LC/UV chromatogram of polyethoxylated nonylphenol (Triton). (Bottom) LD/FT/ICR mass spectrum of Triton, obtained from a single time-domain data set. Reproduced with permission from [77] © 1991 by American Chemical Society.

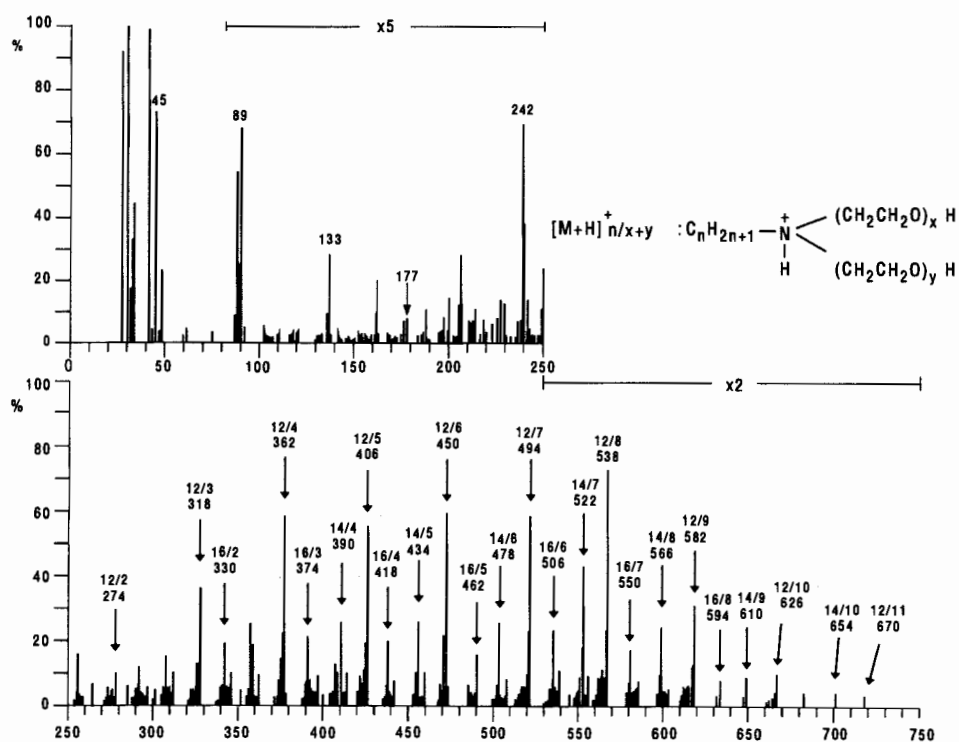
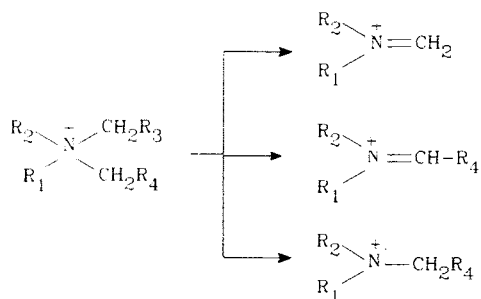


Fig. 14.13. DCI spectrum of an ethoxylated fatty amine. Reproduced with permission from [68] © 1983 by Springer-Verlag.

results from a neutral alkane loss involving hydrogen transfer from the nitrogen with α -cleavage. Alkyl (C_nH_{2n+1}) and alkenyl (C_nH_{2n-1}) ion series are the most abundant fragments for aliphatic and mono-unsaturated amines, respectively, and thus provide structural information. The length of the alkyl chain can be determined from fragment ions separated by 14 Da starting from the ammonium ion and up to the parent ion. Jensen et al. [52] demonstrated that decompositions remote from a charge site are also observed for collisionally activated positive ions. Thus, for octadecylamine the product ions from the $[M+H]^+$ parent show that ions below m/z 90 (the most intense in the spectrum) are formed by the expected fragmentations close to the NH_3^+ functional group. However, the product ions above m/z 90 are due to parallel losses of C_nH_{2n+2} .

Fatty quaternary ammonium salts are the most used cationic surfactants. Their general formula is $R_1R_2N^+(CH_3)_2X$, where R_1 and R_2 are usually long alkyl chains, or R_1 is a long alkyl chain and R_2 is a methyl group. X is Cl^- or Br^- . Their FAB(+) spectra exhibit the quaternary ammonium ion $[L]^+$ as base peak for a single compound or the different intact cations when the compound contains a mixture of homologs. In addition, cluster ions of $[2L+X]^+$ are observed at lower relative intensity, and different fragment ions, which will be discussed further in the context of MS/MS. The use of FAB(-) only showed $[L+2X]^-$ ions, and thus both the anionic moiety and the cationic counterion can be identified.



Scheme 14.3.

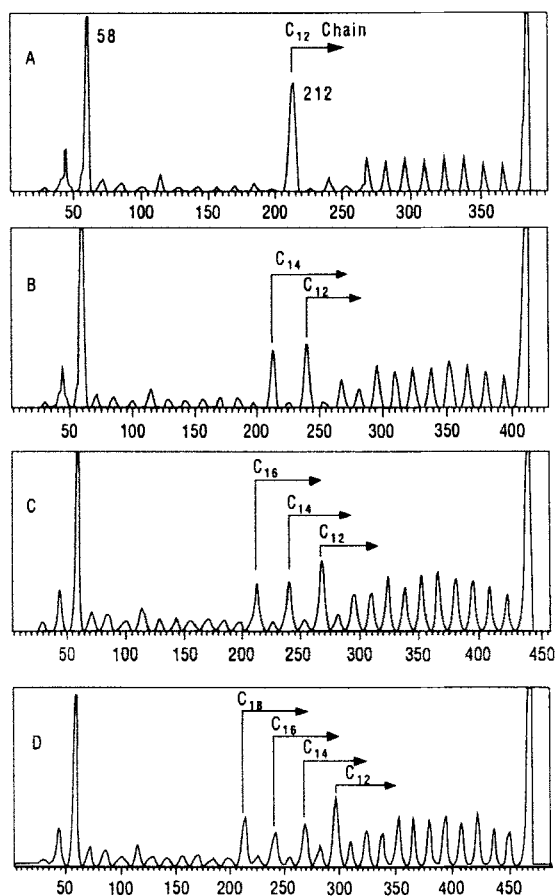


Fig. 14.14. FAB/CAD spectra of positive ions from dimethyldicocoammonium chloride: 9a) m/z 382, the dimethyldidodecylammonium cation; (b) m/z 410, the amine cation with C_{12} and C_{14} alkyl groups; (c) m/z 438, the dialkylamine cations with two C_{14} and $C_{12}C_{16}$ alkyl groups; (d) m/z 466, the dialkylamine cations with $C_{12}C_{18}$ and $C_{14}C_{16}$ alkyl groups. Reproduced with permission from [76] © 1984 by American Chemical Society.

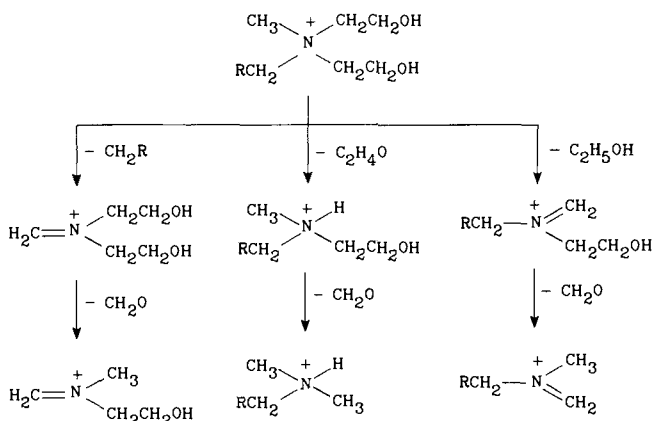
Schneider et al. [68] and Lyon et al. [76] reported the CAD spectra of quaternary ammonium compounds. They found (see Scheme 14.3) that the most abundant ions are caused by: (a) loss of the alkyl group with hydrogen transfer (alkane loss) and (b) loss of an alkyl group with concomitant α -cleavage of a second alkyl group which gives rise to $m/z = 58$, the iminium cation $[(CH_3)_2N^+=CH_2]$. Figures 14.14a–d show the CAD spectra of the positive ions from dimethyldicocoammonium chloride, whose FAB(+) spectrum exhibit a series of intact cations from the different constituents of coconut. Loss of neutral dodecane from the molecular cation gives an intense fragment at $m/z = 212$, and the iminium ion at $m/z = 58$ formed by the loss of both lauryl chains (Fig. 14.14a). The ion at $m/z = 410$ (Fig. 14.14b) could correspond to either the di- C_{13} or the $C_{12}C_{14}$ structures. The CAD spectrum shows losses of the C_{12} and C_{14} groups ($m/z = 240$ and 212 , respectively). The presence of a C_{13} structure is discarded by the absence of a fragment at $m/z = 226$.

The alkylpyridinium cationics also exhibit the quaternary ion as base peak and an intense fragment at $m/z = 80$ that corresponds to the pyridinium ion resulting from loss of alkene from the parent cation.

Polyethoxylated quaternary amines $C_nH_{2n+1}-N^+[(CH_3)(CH_2CH_2O)_x(CH_2CH_2O)_y]X^-$ show, in the positive mode, a complex mixture of homologs with different EO units. The base peak of these compounds $[C_nH_{2n+1}-N^+(=CH_2)(CH_3)]$ results from cleavage along the oxyethylene chains.

CAD spectra of ethoxylated quaternary amines [76] show the fragmentation pathway described in Scheme 14.4. Cleavage, to lose either an ethylene oxide or an alkene are the main fragmentation routes, with preference for cleavage on the EO chain over the long alkyl substituent. Further decomposition of these ions yields fragments at $m/z = 44$ and 58 .

A polyethoxylated quaternary amine, with perfluorobutyrate esters to increase sensitivity at the high molecular weight, has been proposed for FAB calibration [79].



Scheme 14.4.

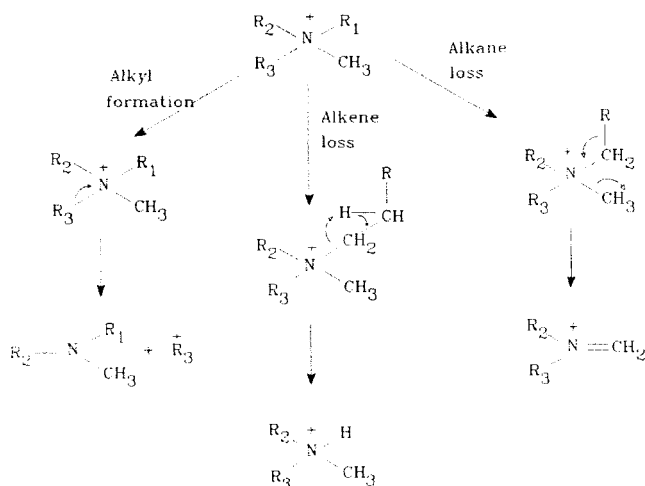
14.2.3.2. Field desorption

Quaternary ammonium salts are the main cationic surfactants studied by FD and FD-CAD [56,68,80–85]. Their FD spectra are dominated by abundant molecular cations $[M]^+$ and clusters of the $[2M + X]^+$ type. Negligible fragment ions are present. The weak signals observed in the spectra are most probably caused by impurities rather than fragmentation processes [56,68,78]. Loss of methyl chloride to give the tertiary amines is a competing process, quite dependent on the heating current in the emitter, as was found by some authors [56,78] but not by others [68]. The intensity of cluster ions decreases at high emitter current, while the intensities of the molecular cation and fragment ions increase [56]. When two or more quaternary ammonium salts are present in a mixture, a hybrid cluster ion $[M_1 + M_2 + X]^+$, together with the intact cations of the different components of the mixture, are present [56,85]. The emitter current required for desorption of a compound is affected by the presence of other compounds in the mixture. Thus, the FD spectra of cationics are less intense and reproducible when anionic surfactants are present [57].

The FD-CAD spectra of quaternary ammonium salts [58,85] are dominated by the product ion formed by loss of the long chain followed by hydrogen transfer. The second most intense product ion involves the alkyl loss followed by α -cleavage in a neighboring alkyl group, leading to the formation of a stable iminium ion $[(CH_3)_2N^+=CH_2]$ at $m/z = 58$. Shen and Al-Saeed [85] observed C_nH_{2n+2} losses caused by dissociation at bond locations remote from the charge center.

14.2.3.3. Other desorption ionization methods

DCI spectra of fatty ammonium quaternary salts have been reported [68,78]. They differ from the FAB spectra because the most abundant peaks are formed either by loss of



Scheme 14.5.

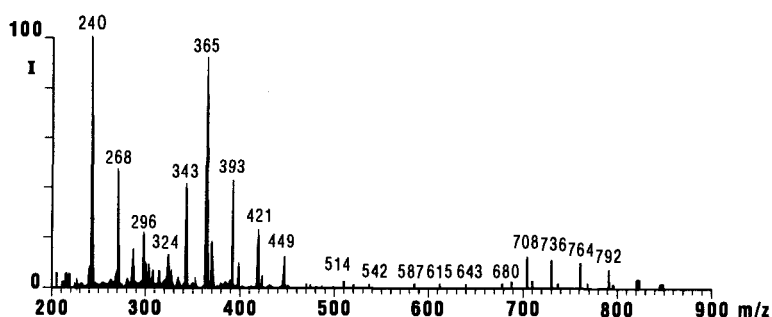


Fig. 14.15. FAB(+) spectrum of cocoamidopropylbetaine. Reproduced with permission from [67] © 1989 by Pergamon Press plc.

methyl chloride followed by protonation (leading to protonated trialkylamines) or by loss of alkyl halide followed by protonation. The quaternary ammonium ion is weak [78] or virtually absent, owing to easy alkane elimination and alkyl elimination with subsequent α -cleavage [68]. Cationic surfactants were largely obscured by the strong response of non-ionics when a mixture of an artificial detergent formulation containing cationic, non-ionic and anionic surfactants was analyzed by positive PD using nitrocellulose as a support [65]. On the other hand, when the support was replaced by Nytran, the cationics dominated the spectrum. A tandem mass spectrometric fragmentation study of cationic surfactants by FAB and LD at various collision energies has been conducted [86]. At low collision energies, the fragmentation pathway favored both the formation of $[C_nH_{2n+4}N]^+$ from alkene elimination and of $[C_nH_{2n+1}]^+$ ions from long chain alkyl fragmentation. When the collision energy was increased the fragmentation pathway shifted towards the formation of $[C_nH_{2n+2}N]^+$ and $[H_2C=N(CH_3)_2]^+$ via an alkane elimination (see Scheme 14.5). The ESI(+) spectra of cationics gave the intact cation as the most abundant peak [66].

14.2.4. Amphoterics

14.2.4.1. Fast atom bombardment

Cocoamidopropylbetaine $[C_nH_{2n+1}-CO-NH-(CH_2)_3-N^+(CH_3)_2-CH_2COO^-]$ [48,67] and *N*-coco- β -aminopropionate [76] are the sole amphoteric compounds studied.

The FAB(+) of cocoamidopropylbetaine (Fig. 14.15) shows intense $[M + H]^+$ and $[M + Na]^+$ ions corresponding to different constituents of coconut. Nevertheless, the base peak at $m/z = 240$ and other fragment ions at $m/z = 268$, 296 and 324 arise from loss of glycine, having the general structure $[R-CO-NH-(CH_3)_2]^+$ with $R = C_{12}-C_{18}$. Different clusters of the $[2M + H]^+$ and $[2M + Na]^+$ type are observed when thioglycerol saturated with NaCl was used as matrix [67]. Other minor fragments are assigned to dimethylaminopropylamide derivatives $[R-CO-NH-(CH_2)_3-N-(CH_3)_2 + H]^+$ of coconut acids present as unreacted materials [48]. Unsuccessful negative FAB spectra are obtained for this amphoteric compound, and only carboxylate anions coming from unreacted fatty acids or

fragments of unreacted intermediate esters are observed [48]. The FAB(+) spectrum of *N*-coco- β -aminopropionate was studied by Lyon et al. [76]. It shows a series of protonated cocoamine molecular ions at $m/z = 258, 286$ and 314 . The difunctionality of the molecule can be revealed by intense ions at $m/z = 258, 280$ and 302 . The first represents the molecule with the amine and the carboxyl having been protonated [$C_{12}H_{25}-N^+H_2-(CH_2)_2-COOH$]. The second ion corresponds to one protonated functionality and the other cationized, whereas the third arises from both cationized functionalities. The product ion spectra from $m/z = 258$ are directed by the nitrogen. Loss of $C_{11}H_{24}$ by α -cleavage and proton transfer yields an ion at $m/z = 102$ and the other cleavage with loss of CH_2COOH gives the fragment ion at $m/z = 198$. A negative FAB spectrum can be obtained being $[M - H]^-$ for C_{12} the base peak of the spectrum.

A fluorinated alkylbetaine has also been used to calibrate high resolution FAB mass measurements [87].

14.2.4.2. Field desorption

Imidazoline-type amphoterics [88,89] and quaternary ammoniumcarboxylates [90,91] show intense molecular ions and fragment ions, whose production can be favored by varying the emitter current. Lactonization can occur, leading to a series of ions due to CO_2 losses. Molecular ions and $M-CO_2$ dominate the spectrum at low emitter currents, while fragment ions are more intense at higher currents.

14.3. ENVIRONMENTAL APPLICATIONS OF DESORPTION IONIZATION METHODS

Analysis of organic pollutants in water by desorption ionization techniques is still limited, with fast atom bombardment and field desorption being the most used. These are mainly qualitative, although the most recent reports deal with quantification. Table 14.1 shows selected examples dealing specifically with water analysis. Isolation techniques for the concentration of surfactants in environmental samples have been reviewed recently [10].

14.3.1. Fast atom bombardment

Righton and Watts [47] used the Wickbold sublation method [92] to study the presence of surfactants in industrial effluents from the manufacture of hair-care products and cationic surfactants, in influent and effluent sewage treatment works, and river and drinking water samples by negative and positive FAB. The comparison of grab and composite samples of an industrial effluent from hair-care products, which were suspected of nitrification inhibition of the activated sludge at the receiving sewage treatment works, showed great variations in the composition of the effluent. Alkyldiethanolamides, trimethylalkyl-, dialkyldimethyl- and alkylpyridinium ammonium salts, sodium alkylsulfates, sodium alkyl ether sulfates, and alkylphenol ethoxylates were identified. In addi-

tion, the FAB(−) exhibited intense $[M - H]^-$ ions of fatty acids or $[M - Na]^-$ ions from their sodium salts. Samples from different stages of the sewage treatment works receiving this effluent showed a decrease through the works, presumably due to the removal of surfactants by the treatment processes, for example by biodegradation. High resolution FAB mass spectrometry and accurate mass measurement allowed the identification of sulfated castor oil as potentially responsible for excessive foaming in a sewage treatment works receiving wastewater from a textile processing factory. Alkylphenol ethoxylates, alkylbenzene sulfonates and quaternary ammonium salts were also identified. No conclusions could be made about which compound or compounds were directly responsible for the foaming. However, alkylbenzenesulfonates, alkylphenol ethoxylates and alkylsulfonates were identified as causing foaming problems in other textile processing plants and printworks effluents and it seems that these compounds were almost certainly responsible.

Dimethyldiallowammonium chloride has been identified in wastewater using liquid-liquid extraction and FAB-MS [20]. Tributyl phosphate, non-ionic surfactants such as polyethoxylated nonylphenols, octylphenols, glycerin, alcohols, and anionic surfactants such as alkylbenzenesulfonates have been characterized as additives present in commercial sulfonated azo dyes, which are common products dumped by textile industries into the rivers [93]. Cationic and non-ionic surfactants have also been identified in urban wastewaters and coastal receiving aquatic systems [94,95]. LAS and dialkyltetralinsulfonates have been identified in sewage contaminated groundwater by FAB(−) and accurate mass measurements [49].

Analysis of surfactants has been performed for raw and drinking water of Barcelona (N.E. Spain) by adsorption on granular activated carbon (GAC) of the same type as used in the water works plant or on XAD-2 [20,22,67,96–99]. The organic extracts after a classical fractionation scheme are divided into acidic and (base + neutral) compounds. The extracts are fractionated by reversed-phase LC and further analyzed by FAB. The main identified surfactants are non-ionic (polyethoxylated nonylphenols and alcohols, poly(ethylenepropylene) block copolymers) and anionics (alkylbenzenesulfonates and lauryl ether sulfates), with minor amounts of cationics (quaternary ammonium salts, such as dimethyldiallowammonium chloride and dimethyl-laurylbenzylammonium chloride). Figure 14.16 shows the FAB(+) spectra of some real raw and tap water LC fractions. Other interesting compounds present are those related to known biotransformation products from alkylphenols in water such as the acidic metabolites of nonylphenols $[C_9H_{19}-C_6H_4-(OCH_2CH_2)_n-OCH_2-COOH]$, which were previously identified by GC/MS with a low degree of polyethoxylation [19,22]. Brominated nonylphenols $[C_9H_{19}-BrC_6H_3-(OCH_2CH_2)_n-OH]$ and their acidic metabolites $[C_9H_{19}-BrC_6H_3-(OCH_2CH_2)_n-OCH_2-COOH]$ which were detected only in tap water, are formed in water works plants by the treatment of raw water which has high contents of bromide ion (i.e. from salt mine discharges) and surfactants.

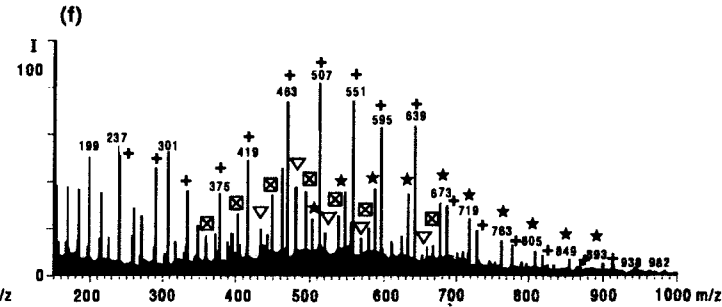
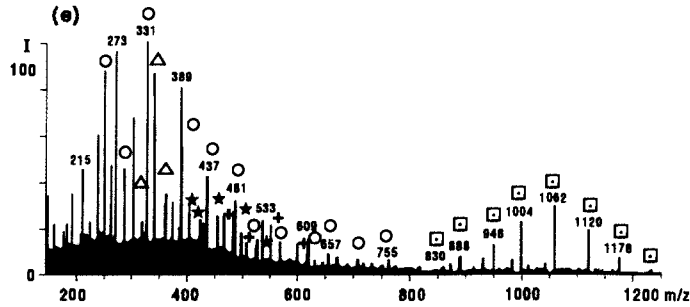
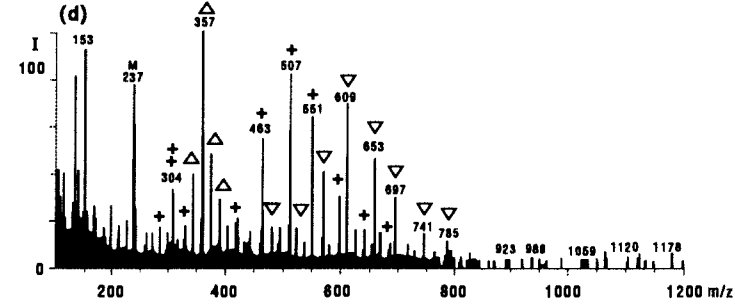
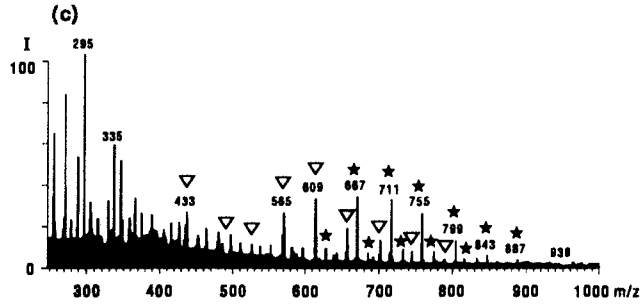
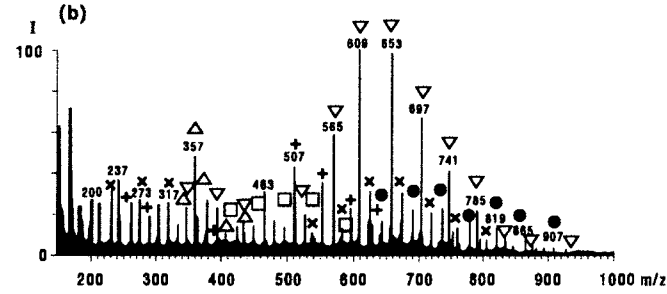
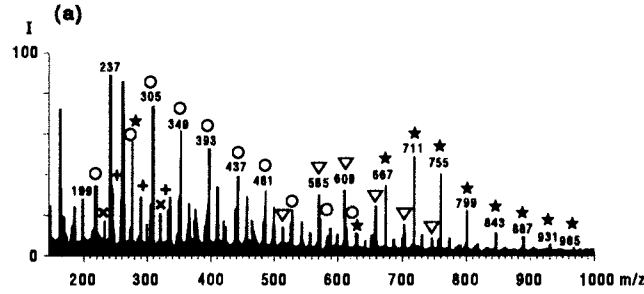
Bromoalkylphenol ethoxylates give $[M + Na]^+$ ions for ^{79}Br and ^{81}Br of each oligomer, with the characteristic isotopic pattern of brominated compounds. Acidic metabolites of alkylphenol ethoxylates present in acidic fractions, showed as base peak the fragment at $m/z = 117$ that corresponds to $[(CH_2CH_2O)-OCH_2-COOCH_3]^+$, and abundant $[M + Na]^+$ ions. Other fragments due to α,α -dimethyl- and α -methyl- α -ethyl cleavage of the alkyl chain [70] were also observed in their EI spectra [16,21]. A comparison between

TABLE 14.1

DETERMINATION OF SURFACTANTS IN WATER BY DESORPTION IONIZATION METHODS

Compound	Sample	Isolation technique	Identification	Ref.
Anionics, non-ionics, cationics	Industrial effluents, effluent sewage treatment works, river, tap water	Sublation	FAB(+),(-)	[47]
Dimethylditallow ammonium chloride	River water	Liquid-liquid extraction	FAB(+)	[20]
LAS, nonylphenol ethoxylates, quaternary amines	Urban wastewaters, seawater sediments	XAD-2; liquid-liquid extraction	FAB(+),(-)	[94,95]
LAS and DATS	Sewage groundwater	SPE on C ₂ and anion exchange	HPLC/FL and FAB(-)	[49]
Anionics, non-ionics cationics, acidic metabolites and brominated derivatives of alkylphenols	River and tap water	Carbon adsorption, XAD-2 HPLC fractionation	GC/MS, FAB(+),(-)	[22,67, 96-99]

Acidic metabolites of alcohol ethoxylates	River and tap water	Carbon adsorption, XAD-2	FAB(+), FAB/MS/MS	[70]
Acidic metabolites of quaternary amines	Ozonated water	Liquid-liquid extraction	GC/MS, FAB(+)	[103]
Degradation of poly-ethoxylated nonyl-phenols	Ozonated water	Liquid-liquid extraction	HPLC, FAB	[104]
Lauryldimethyl- and dimethylditallow ammonium chloride	Sewage influent, effluent river water	Evaporation, extraction of dried extract, alumina adsorption	Quantitative FAB(+)	[105]
LAS, ABS	Sewage, river water	SPE on C-18 disks; SAX and C-18	Quantitative FAB(–)	[51]
Alkylphenol ethoxylates	River water	Concentration on HPLC column	HPLC/UV and FD/MS	[115]
LAS, quaternary amines	River water	Sublation	FD/CAD/MS	[116]
Quaternary amines, nonylphenol ethoxylates	River and drinking water	Freeze-dry or SPE on XAD-2 and HPLC	FD and FAB	[117,118]
Nonylphenol ethoxylates, dimethylditallow ammonium chloride	River dye-away test	Evaporation and dissolution in chloroform	FD	[119,120]
Alcohol and nonyl ethoxylates	River water	XAD-4, sublation	Quantitative FD	[121]



GC/MS and FAB demonstrated that although the same non-ionic surfactants were identified, GC/MS is limited to compounds with a low degree of ethoxylation, whereas FAB showed a higher range of oligomers [22].

One major disadvantage of FAB arises from compounds that can suppress the ionization of others present in the samples and in the matrix background. However, when two or more compounds give the same series of peaks, reliable identification can be achieved by comparison of both the negative and positive FAB, by tandem mass spectrometry or accurate mass measurement. In this last technique, external or internal standards can be used, but more accurate mass measurements are obtained with internal standards. The main problem then is finding the most suitable standards, which may be difficult and time consuming. Any standard should cause no suppression of the unknown(s), nor be suppressed by them, and give steady signals over a wide mass range. With this aim, mixtures of different poly(ethylene) glycols [100,101], an amphoteric surfactant [87], amino acids [102] and non-ionic surfactants [69] have been employed. Ventura et al. [70] used their own nonylphenol ethoxylates present in the (base + neutral) fractions, and their acidic metabolites present in the acidic fractions, as internal standards for accurate mass measurements of unidentified compounds in water extracts, thereby avoiding manipulation of the sample.

The use of MS/MS is necessary for the unequivocal identification of compounds which show the same behavior under FAB conditions and where accurate mass measurement is unsuitable for distinguishing them (i.e. same molecular formula). An example of the application of FAB/MS/MS is the unequivocal identification of acidic metabolites of polyethoxylated alcohols in water extracts [70]. The FAB(+) spectrum of an acidic fraction (analyzed as the methyl esters) of tap water (see Fig. 14.17) showed a series of peaks, with 44-Da spacing that corresponded to different oligomers of non-ionic surfactants. It was observed by varying the degree of polyethoxylation (n) and the length of the alkyl chain (m), that polyethoxylated alcohols [$C_mH_{2m+1}-(OCH_2CH_2O)_n-OH$], acidic metabolites of the type found for alkylphenols [$C_{m-1}H_{2m-1}-(OCH_2CH_2)_{n-1}-OCH_2-COOH$], and the common metabolites described for polyethoxylated alcohols [$HOOC-(CH_2)_{m-1}-(OCH_2CH_2)_n-OH$], gave the same series of peaks in FAB. The presence of polyethoxylated alcohols was rapidly disproved by accurate mass measurement. Comparison of the MS/MS spectra of both possible acidic metabolites, which have the same molecular formula, and the unknown present in the tap water (see Fig. 14.18) allowed the confirmation of the presence of metabolites of polyethoxylated alcohols which had been produced by carboxylation of the ethoxy chain instead of the alkyl chain.

Ozone is widely used in Europe and increasingly in the United States, as an alternative to chlorine for the disinfection of drinking water. It has the advantage of eliminating or

Fig. 14.16. FAB(+) spectra of real water samples. (a,b) LC fractions of raw water. (c,d) LC fractions of raw water entering the waterworks plant. (e,f) LC fractions of drinking water. Key: ○, polyethyleneglycol; ∇, nonylphenol ether sulfates; ★, poly(ethoxypropoxylated)nonylphenols; +, polyethoxylated nonylphenols; ×, polyethoxylated *i*-octylphenols; Δ, alkylbenzenesulfonates; □, polyethoxylated dodecylphenols; M, matrix; ‡, dimethyl laurylbenzylammonium chloride; ☐, poly(ethylenepropylene) block copolymers; ☒, lauryl ether sulfates; ☆, polyethoxylated bromononylphenols. Reprinted with permission from [67] © 1989 by Pergamon Press plc.

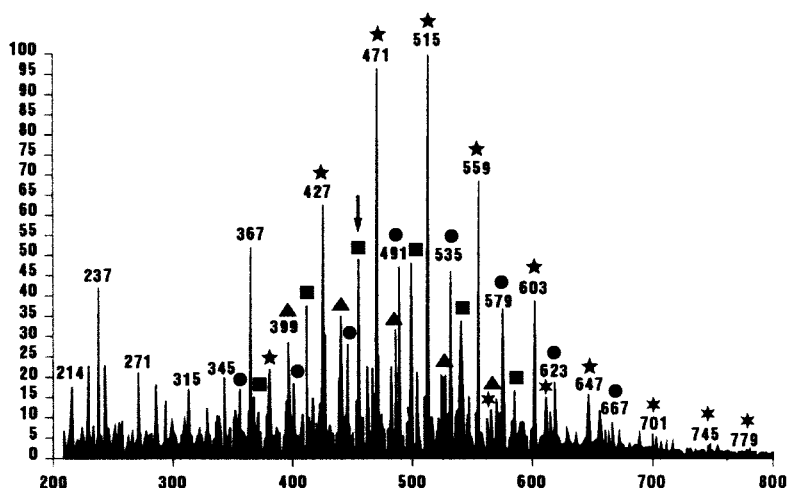


Fig. 14.17. FAB(+) spectrum. Acidic fraction (as methyl esters) of tap water. $[M + Na]^+$ peaks. ■, Acidic metabolites of polyethoxylated nonylphenols; ★, acidic metabolites of polyethoxylated bromononylphenols; ★, ■, ▲, polyethoxylated alcohols or their different acidic metabolites. Reproduced with permission from [70] © 1991 by American Chemical Society.

reducing undesirable chlorination disinfection by-products such as the trihalomethanes. However, the characterization of oxidation products from the reaction of ozone with substances present in raw water is still limited. Corless et al. [103] characterized the reaction products from ozonation of a quaternary ammonium surfactant (dimethyldiallow-ammonium chloride) by FAB. They found that the ozonated water exhibited evidence of the consumption of unsaturated components of the cationic surfactant, while the saturated ones remained constant. Accurate mass measurements by FAB indicated that the reaction products were quaternary ammonium carboxylic acids (see Fig. 14.19). No quaternary ammonium aldehydes were identified, suggesting that their oxidation to the carboxylic acids was complete within the 30 min. ozonation period. The proposed pathway for the reaction between the ozone and the unsaturated components of the cationic surfactant is consistent with a 1,3-dipolar cycloaddition reaction of ozone to a carbon-carbon double bond. The ozonation products of nonylphenol ethoxylates at different pH and over different periods has been studied by LC and FAB [104]. The authors suggested that the reaction occurs essentially through a hydrogen abstraction at one of the ethoxylated units followed by depolymerization.

Quantification of surfactants by FAB has been performed [51,105–108]. The analyte surface activity differences, which are strongly dependent upon the alkyl chain lengths in a series of homologous surfactants, often significantly affect the relative response in FAB [109–111]. This leads to the selective detection of the more surface-active components in mixtures and precludes the use of homologs as internal standards for quantitative FAB [105].

The quantitative determination of cationic surfactants at the ppb level in aqueous environmental matrices such as sewage influents and effluents and river water, has been

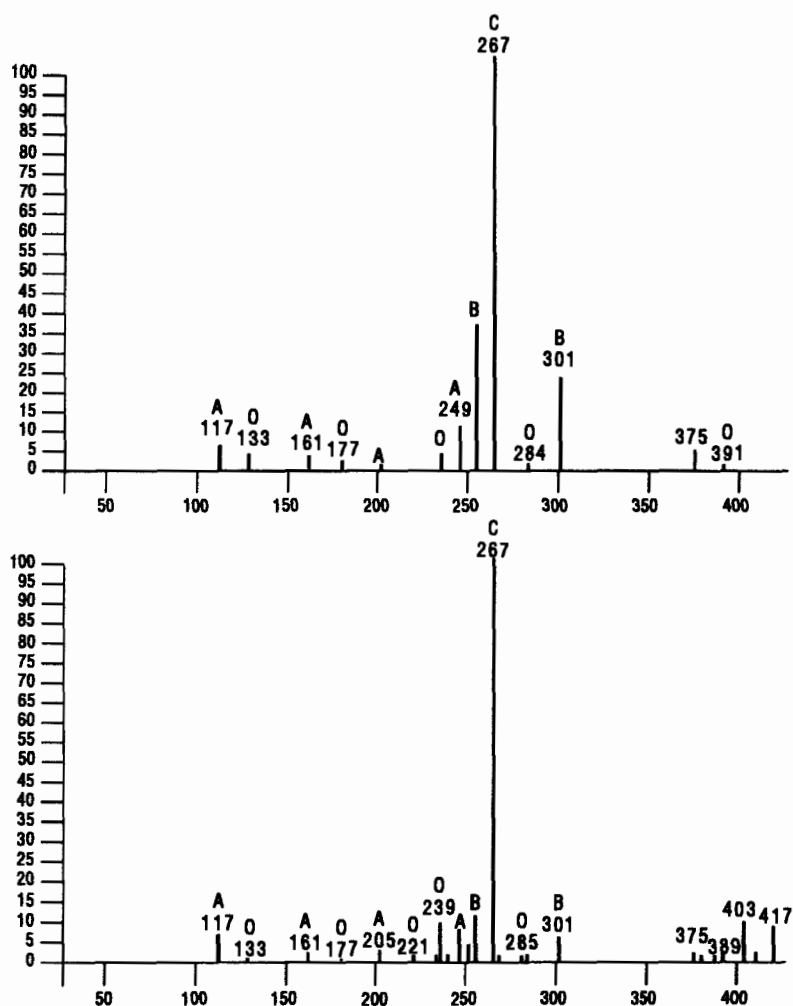


Fig. 14.18. (Top) MS/MS spectrum of the [(lauryloxy)tetraethoxy]acetic acid. Parent ion $[M + H]^+ = 435$. (Bottom) MS/MS spectrum of an unknown compound present in tap water (see Fig. 14.16, marked with an arrow). Same parent ion $[M + H]^+ = 435$. Reproduced with permission from [70] © 1991 by American Chemical Society.

evaluated using low resolution full scan (FS) and high resolution continuum acquisition (CA) methods [105]. These have been applied to the quantitation of lauryltrimethylammonium chloride (LTMAC) and ditallowdimethylammonium chloride (DTDMAC). FAB-MS calibration curves were obtained by plotting the ratio of analyte response to the internal standard response (D_3 -LTMAC and D_3 -DTDMAC, respectively) as a function of the corresponding ratio of concentrations. The calibration curves were linear over the full range of concentrations, except for dimethylditallowammonium chloride which deviated from linearity when the ratio DTDMAC/ D_3 -DTDMAC is higher than 10 (see Fig. 14.20).

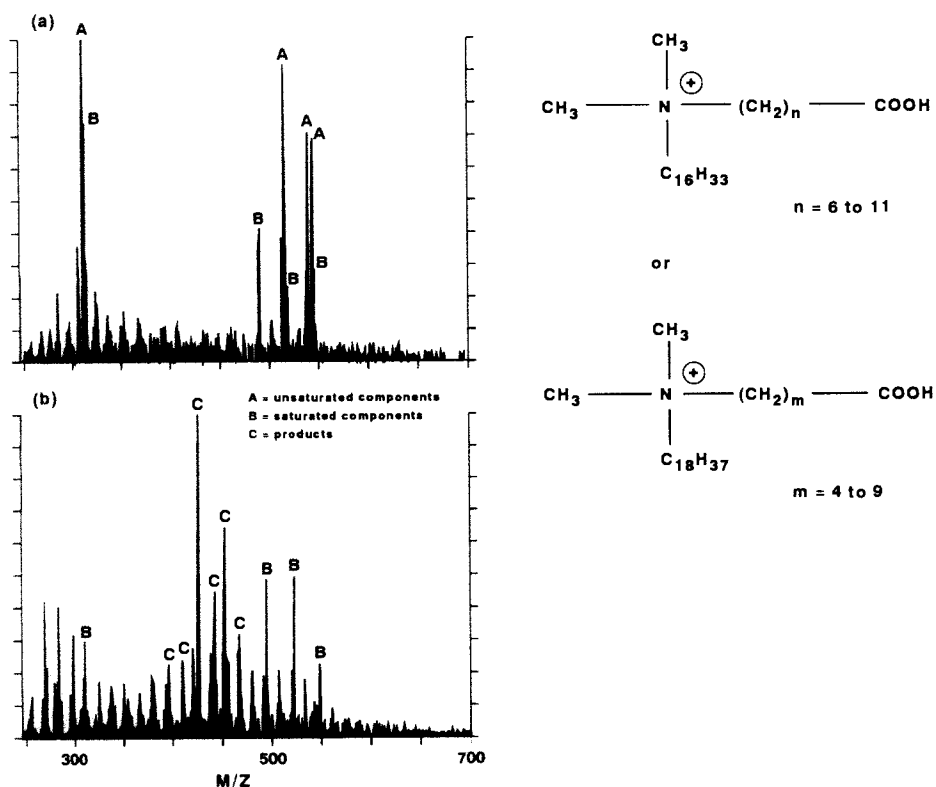


Fig. 14.19. FAB(+) mass spectra of a quaternary amine surfactant (a) before, and (b) after ozonation, and their possible products. Reproduced with permission from [103] © 1991 by Pergamon Press plc.

The role of surfactant hydrophobicity on the FAB-MS response was also studied. It was found that although DTDMAC is much more hydrophobic, the signal of LTMAC was not suppressed in glycerol even when high levels of DTDMAC or anionic LAS were present to diminish surface activities. Different results were obtained by changing the matrix showing that the choice of the FAB matrix has a critical influence on the observed spectra. The two methods used gave comparable quantitative results for both cationic surfactants in the 10–500 ppb range but the FS is less reproducible than CA. On the other hand, the advantage of the FS method is that it allows simultaneous quantitation of selected surfactants. The CA method is the method of choice for quantitation of a single component, especially below 10 ppb and above the 1000 ppb level.

Wernery and Peake [106] investigated the effect of surface activity differences between LAS homologs on FAB-MS quantifications. They found that at lower total LAS concentrations, the abundance of C₁₄-LAS is enhanced relative to the shorter chain homologs. This trend is reversed as the LAS concentration increases, until the relative abundance of the C₁₀-LAS is greater than the C₁₂- and C₁₄-LAS. This reverse discrimination has been attributed to the formation of micelles by the longer chain and subsequent

depletion of the more surface-active component from the surface [112]. The relative abundance of LAS homologs was also highly dependent on both the LAS concentration and the data acquisition time. Therefore, accurate analysis is impossible unless one brackets the LAS homologs with appropriate internal standards. The use of a single deuterated internal standard (D_7C_{12} -LAS) showed skewing of the distribution of LAS homologs. Nevertheless, when the three stable isotope internal standards (D_7C_{10} -, C_{12} -, and C_{14} -LAS) were employed, the differences in surface activity of the LAS homologs were compensated, and accurate mass measurements were feasible.

Recently, another approach for a quantitative method for the analysis of LAS in samples from a wastewater treatment plant and from river water has been conducted by Borgerding and Hites [51], using continuous-flow FAB (CFFAB) and tandem mass spectrometry. It has been reported that CFFAB reduces surface activity effects [113] and is more sensitive [114] than FAB. Their method was based on scanning the parent ions of $m/z = 183$, which is a product ion common to all LAS subjected to CAD (see Fig. 14.3). In addition, the parent-ion scan method can be used to selectively determine ABS homolog concentrations, even in the presence of LAS. Thus, the ABS concentrations can be determined by scanning the parent ions of $m/z = 197$ which is the characteristic product ion (see Fig. 14.3) not observed in LAS. Nevertheless, as the product ions of both ABS and LAS contain the ethylene-substituted benzenesulfonate at $m/z = 183$, the quantification of LAS requires confirmation of the absence of any ABS that could skew the LAS measurement. A quantitation based on peak areas is linear from 1 to at least 100 ng, with a practical limit of detection being about 1 ng injected, which corresponds to $0.5 \mu\text{g/l}$ for a 1-l sample. The method that uses flow injection, requires no chromatography and avoids the complexities of the relative ionization efficiencies of the LAS homologs and the concentration dependence observed by other authors in FAB analysis [106].

No quantitative analysis by FAB of non-ionic and amphoteric surfactants in environmental samples has been reported, to the best of our knowledge.

14.3.2. Field desorption

Otsuki and Shiraishi determined alkylphenol ethoxylates in river water samples by field desorption [115]. Surfactants were adsorbed on a C-18 column and eluted by gradi-

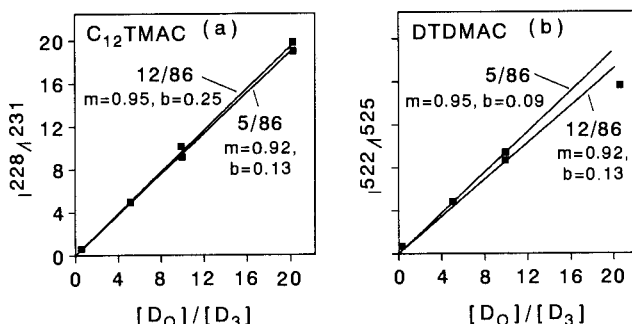


Fig. 14.20. Typical FAB/MS calibration data for LTMAC and DTDMAC. The internal standard level is 50 ppb. Reproduced with permission from [105] © 1988 by American Chemical Society.

ent elution using mixtures from water to 100% methanol, which allowed the separation of octyl-, nonyl- and dodecylphenyl ethoxylates. A collected fraction was subjected to analysis by FD and showed an intense series of $[M]^+$ peaks, spaced by 44 Da, corresponding to nonylphenyl ethoxylates (from $n = 5-13$). The authors suggested that samples containing more than 1 mg/l of surfactants could be analyzed directly. On the other hand, the analysis of such complex mixtures required the integration of each FD mass spectrum from each repeated scan during the emitter current program in order to give reproducible FD spectra.

Surfactants from river Rhine water extracts, obtained by sublation, were identified by FD/CAD [116]. Figure 14.21 shows the FD spectra obtained at three different emitter heating currents, that allowed the identification of non-ionic surfactants (at 5 mA), quaternary ammonium salts (20 mA) and alkylbenzenesulfonates at 23 mA. Non-ionic surfactants showed different $[M + Na]^+$ ions, corresponding to several degrees of polyethoxylation that were assigned to dodecylphenol ethoxylates but their unspecific fragmentation

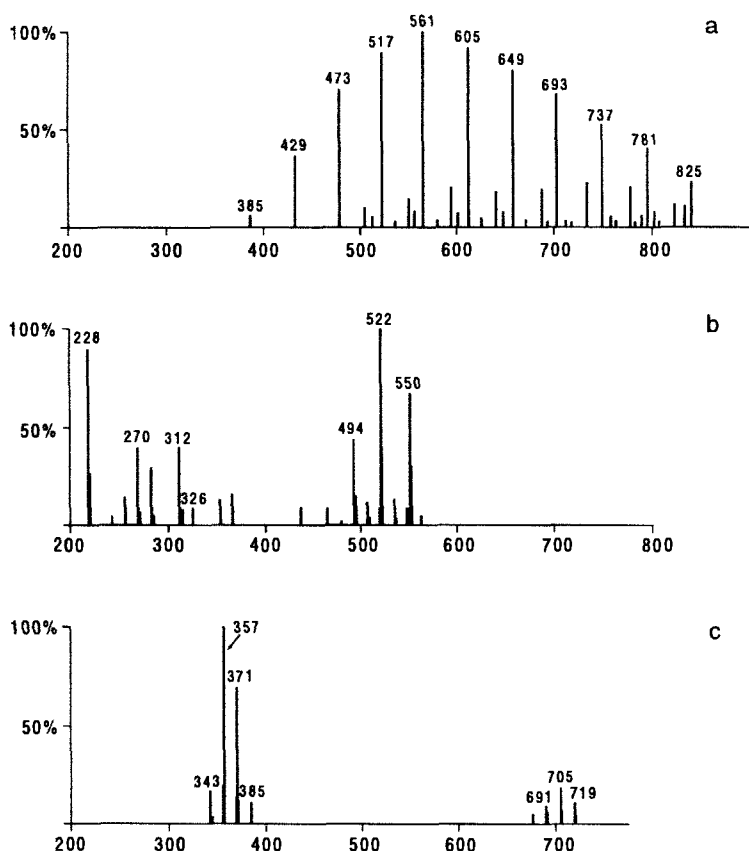


Fig. 14.21. FD spectra of a water sample from the river Rhine. Emitter heating current was (a) 5 mA, (b) 20 mA and (c) 23 mA. Reproduced with permission from [116] © 1984 by American Chemical Society.

under CAD conditions could not be used to characterize their structures. Some problems were observed when excess of salts from the extraction procedure were present, making it difficult to obtain good FD(+) spectra for anionic surfactants. The problem was solved by using negative FD. The method was very sensitive, allowing full spectra from only 1 ng of cationics, while 1 μg and 100 ng, respectively, were necessary for anionic and non-ionic surfactants. The results showed that the proper choice of the emitter heating current partially separates the three type of surfactants.

Non-volatile organic compounds from British and German rivers were analyzed by FD and FAB [117,118]. Freeze-drying or XAD-2 resin adsorption and further separation by normal or reverse-phase LC of the total extracts were used as isolation techniques. The LC fractions were subjected to FD and FAB, with the FD spectra generally being more informative. The authors were able to characterize among others, uron herbicides, pesticides, pharmaceuticals, and non-ionic (alkylphenol ethoxylates) and cationic (quaternary ammonium salt) surfactants.

The primary biodegradation of non-ionic and cationic surfactants in surface waters has been monitored by FD [119,120]. Figure 14.22 shows the FD spectra of the progress of biodegradation of a nonylphenol ethoxylate, using octylphenols as standards (unmarked peaks in the figure). Dinonylphenol ethoxylates, marked with arrows, are probably impurities from the commercial product. At the beginning of the experiment (day 0), the spectrum was dominated by peaks from $n = 4-8$. After 7 days, the FD spectrum changed, and the most intense peak was nonylphenol diethoxylate. Higher homologs disappeared and the dinonylphenols remained unchanged. Finally, the FD spectrum at 44 days showed the homolog with two ethylene oxide units almost completely biodegraded, while the dinonylphenols still persisted. The results demonstrated that biodegradation of the homolog with two EO units is a fast process, while further biodegradation occurs slowly. A similar approach was carried out with a cationic surfactant, such as distearyldimethylammonium chloride. The authors found that primary biodegradation starts after approximately 14 days and is not complete after 50 days, showing that primary biodegradation of cationic surfactants is considerably slower than that of nonylphenol ethoxylates.

Quantification of polyethoxylated alcohols and nonylphenols in river water has been performed by FD [121]. The FD spectra of standards produced $[M + H]^+$, $[M + Na]^+$ and cluster ions of the $[M_1 + M_2 + H]^+$ type and were affected by the emitter current, the amount of sample loading, and the presence of salts. The XAD-4 adsorption and sublation were used as isolation techniques. The FD spectra of the samples from the sublation methods were dominated by $[M + Na]^+$ ions. The use of a stable-isotope-labeled internal standard was necessary for quantification by FD because of fluctuations in the ion current. The authors employed tetradeuterioocta(oxyethylene) dodecyl ether as internal standard. Calibration curves showed a good linear relationship between the amount of sample and the added internal standard, with the optimum sample size for the determination being 3–5 μg up to 10 ng. The intensities of each oligomer relative to the internal standard were determined by using the intensities of $[M + Li]^+$ ions in the integrated FD spectrum of an equimolar mixture of polyethoxylated lauryl alcohol, which showed an increase in sensitivity when the number of EO units increased. The concentrations of the surfactants were calculated on the assumption that the sensitivities do not depend upon the number of carbon atoms in the alkyl group.

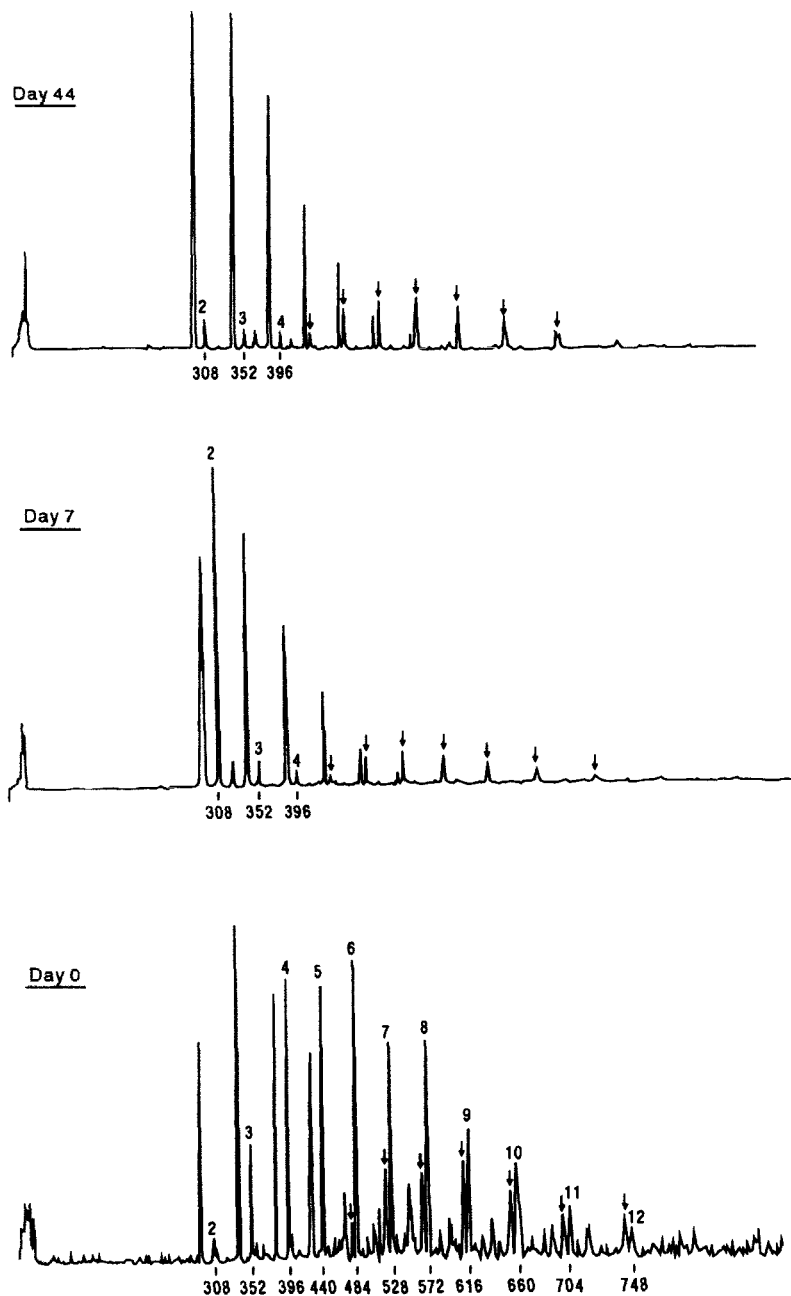


Fig. 14.22. Biodegradation of a nonylphenol ethoxylate in surface water. Field desorption spectrum obtained after (a) 0, (b) 7 and (c) 44 days. Peaks belonging to the nonylphenol ethoxylate are marked by a number which represents the number of ethylene oxide units. Dinonylphenol ethoxylates are indicated by an arrow. Unmarked peaks belong to the standard. Reproduced with permission from [119] ©1987 by Springer-Verlag.

14.4. CONCLUSIONS

The analysis of surfactants by desorption ionization methods has been demonstrated to be an interesting alternative to more conventional methods that are usually more time consuming and restricted to volatile or derivatized compounds. Commercial surfactants have been well characterized by all the desorption ionization methods, although their identification in environmental samples has mainly been performed by FD and FAB. Most studies of surfactant analysis in water are qualitative, but the most recent reports deal with quantification. Accurate mass measurements and MS/MS techniques have been used to characterize biodegradation products.

However, desorption ionization methods are seldom applied in environmental analysis, although non-volatile compounds that can represent as much as 80% of the total organic fraction have been little studied. Future MS applications in environmental analysis will require the use of these methods, especially when they become available to most laboratories.

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Chapter 15

LC-MS interfacing systems in environmental analysis: application to polar pesticides

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15.1. INTRODUCTION

The widespread application of pesticides in agriculture has led to their ubiquitous presence in the environment. Since their occurrence even at low concentrations can be a serious threat to the quality of human life, pesticides – and in principle, also their degradation products – have to be monitored in, e.g. water and food at trace levels of, typically, 0.1 µg/l for water and 1 mg/kg for foodstuff. To meet these requirements, in many cases capillary gas chromatography (GC) combined with selective detectors such as the nitrogen-phosphorus, electron-capture and flame-photometric detectors or a mass spectrometer, is used. However, many pesticides currently in use are not suitable for direct GC analysis due to their high polarity, thermolability and/or low volatility. Separation of these compounds by means of reversed-phase liquid chromatography (LC) is more appropriate, and a large number of studies on this subject has been published over the years (for a re-

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view, see [1]). Detection is usually carried out by ultraviolet (UV) or electrochemical detection, and is sometimes combined with postcolumn reaction/extraction detection. Unfortunately, these detection methods do not provide sufficient selectivity and/or sensitivity for the determination of pesticides in environmental samples, and increasing attention has therefore been devoted to the on-line coupling of LC with mass spectrometry (MS).

Over the years GC-MS has proven to be a particularly powerful technique [2]. However, coupling LC with MS causes a number of technical problems because MS demands the maintenance of a high vacuum in the analyzer region, while in conventional-size reversed-phase LC (2–4.6 mm i.d. columns) liquid flow rates of 0.5–2 ml/min are used, resulting in a mass flow which is difficult for a mass spectrometer to accommodate. Despite this apparent incompatibility, several interfaces for the coupling of LC and MS have been designed over the years (see, e.g. [2–5]).

LC-MS is predominantly applied in biomedical research, mainly for the determination of drugs and high-molecular-weight compounds such as proteins and DNA adducts. However, in recent years increasing attention has been paid to the development of LC-MS procedures for environmental analysis, and several reviews on this subject have been published [6,7].

In this review, recent developments in the use of LC-MS in environmental analysis, in particular for the determination of polar pesticides, are outlined. Attention will be paid to the various interfacing methods used, such as direct liquid introduction (DLI), thermospray (TSP), particle beam (PB) and to atmospheric pressure ionization (API) techniques, such as ion-spray and atmospheric pressure chemical ionization (APCI). The moving belt interface, which played a prominent role in the early days of interface development, is not discussed because of its inherent incompatibility with conventional-size reversed-phase LC. Pesticides to be discussed are the phenylurea herbicides, triazines, carbamates, organophosphorus insecticides, chlorophenols and chlorinated phenoxy acids.

In the discussion regarding the performance of the various systems, due attention is given to the detection limits that can be obtained. Yet detectability is not the only requirement to be met in environmental analysis: in addition to trace-level determination, confirmation of the identity of target compounds and the identification of non-target analytes are important issues. Appropriate attention is therefore also devoted to these aspects.

15.2. LC-MS INTERFACES

15.2.1. Direct liquid introduction interface

Along with the moving belt [8], direct liquid introduction (DLI) was one of the first techniques used to couple an LC system to a mass spectrometer [9]. The DLI interface is as simple as the name implies: the LC effluent is introduced directly into the ion source of the mass spectrometer via a properly constructed connective capillary. This imposes limitations on the flow rates that can be used; flow rates higher than about 50 μ l/min necessitate the use of split-flow techniques in order to maintain the vacuum in the ion source. Unfortunately, loss of analyte and, consequently, analyte detectability, is an inherent dis-

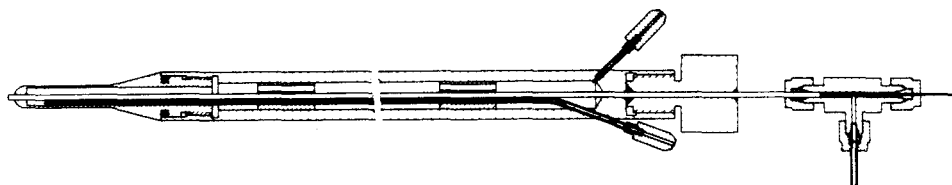


Fig. 15.1. Schematic diagram of a helium jet direct liquid introduction interface.

advantage of split-flow techniques. DLI interfaces are therefore not suitable for combination with conventional-size LC, and micro-LC systems (0.3–1 mm i.d. columns; flow, 10–50 $\mu\text{l}/\text{min}$) should be used instead. Various DLI interface designs for the coupling of micro-LC with MS have been described [10–12]. In this chapter, attention is focused on the helium jet DLI interface [13], mainly because of several interesting studies dealing with on-line trace enrichment by means of precolumn concentration, signal enhancement by using chlorinated organic modifiers and postcolumn extraction techniques.

The helium jet interface (see Fig. 15.1) consists of a 0.1 mm i.d. fused silica capillary, through which the LC column effluent enters the MS ion source. The capillary is coaxial with a 0.3 mm i.d. stainless-steel capillary, and extends approximately 0.5 mm past the end of the stainless-steel capillary into the MS. A helium jet (10–20 ml/min), which flows around the fused silica capillary and into the MS source, is used to nebulize the vaporizing liquid exiting from the fused silica capillary directly into the heated MS source. If necessary, cooling of the DLI interface probe is possible. With this system, 0.7–1.0 mm i.d. LC columns and flow rates varying from 10 to 100 $\mu\text{l}/\text{min}$ can be used.

Ionization of the analytes takes place via chemical ionization (CI), with the solvent vapour acting as reagent gas for CI. Electron impact (EI) ionization is not possible with the DLI interface. The helium jet interface can be used to analyze thermolabile compounds because very little heat is applied before introduction of the LC effluent into the MS.

15.2.2. Thermospray interface

The thermospray technique was developed by Vestal and co-workers [14] in an elaborate search for an interface that would enable the coupling of conventional-size LC and MS detection. In the TSP interface – sometimes regarded as “hot” DLI – two processes take place: (i) a fine nebulization and nearly complete desolvation of the LC effluent before it enters the vacuum region of the MS; and (ii) a “soft” chemical ionization of non-volatile and thermolabile analytes. In the TSP interface (Fig. 15.2), after leaving the column, the LC effluent is transported through an electrically heated capillary. Upon exiting from this capillary, the effluent is transformed into a supersonic jet of vapour and droplets and sprayed into a heated chamber where further desolvation and vaporization take place. Ions formed in the spray are extracted into the mass analyzer region through an ion exit cone positioned perpendicular to the direction of the jet, while the neutral molecules go to a cold trap connected to a vacuum pump. In some cases a repeller electrode is used to assist the ion extraction procedure.

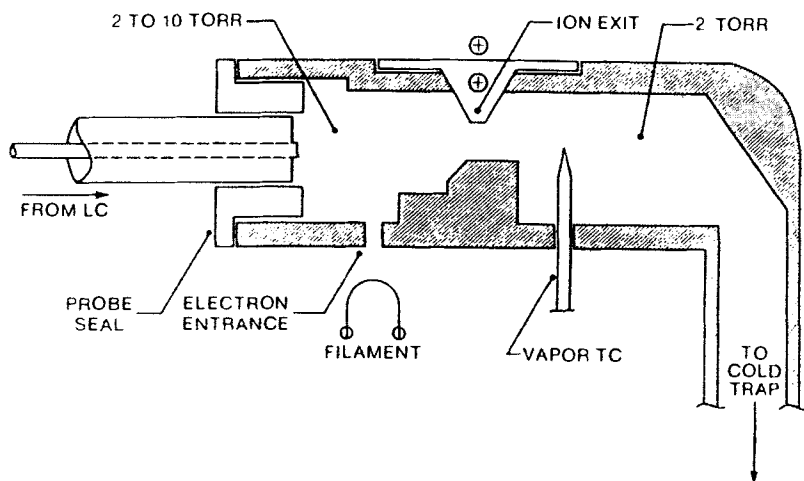


Fig. 15.2. Schematic diagram of a thermospray interface.

Ionization of the analytes can be effected in different ways. In the filament-off mode, ionization takes place under the influence of salts such as ammonium acetate or formate which have been added to the LC effluent, without the aid of an external source. In the filament-on mode EI ionization of the mobile phase constituents is effected by the filament, with subsequent chemical ionization of the analytes. In the discharge mode, ionization takes place under the influence of an electrical discharge. The precise mechanism of the ionization processes taking place in the TSP interface has been the subject of some controversy, but agreement has been reached on a model in which gas phase ionization plays the main role, with some ions presumably being formed by ion evaporation processes [15].

The TSP interface can definitely be regarded as a breakthrough in interfacing conventional-size LC with MS. An additional advantage is the commercial availability from several manufacturers which has rapidly led to the widespread use of LC-TSP-MS. A major drawback is the lack of structural information that can be derived from the mass spectra obtained by TSP-MS. Because of the soft chemical ionization, fragmentation of the analytes is limited, so that identification of unknown compounds, which is often required in environmental analysis, is virtually impossible.

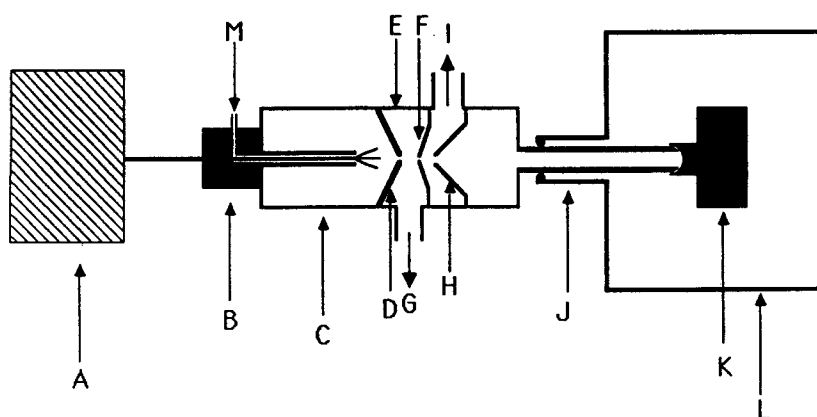
15.2.3. Particle beam interface

Despite the good results obtained with LC-TSP-MS for the determination of molecular weights and the identification of target compounds, to increase the structural information content, there clearly still is a need for an interface which will enable the coupling of conventional-size LC with MS using EI ionization. The PB interface, developed by Willoughby and Browner [16], meets these criteria and is now commercially available. In the PB interface, a nearly complete separation of mobile phase constituents and analytes

is effected, resulting in a beam of dry particles entering the ion source where, apart from CI, EI ionization can occur.

A schematic diagram of a PB interface is shown in Fig. 15.3. Firstly, an aerosol is generated by pumping the LC effluent through a small (25 μ m) orifice, so that a liquid jet is formed which contains droplets of nearly uniform size. The droplets are dispersed by a flow of helium to prevent them from clustering. Subsequently, vaporization of the liquid jet takes place in the desolvation chamber, at atmospheric pressure. The mixture of solvent vapour, helium and analytes is then led through a nozzle, and supersonic expansion results in the formation of a gas jet containing the analytes. Because of the high mass of the analytes relative to that of the solvent molecules, diffusion of the analyte molecules from the jet axis is slow. By placing a skimmer in the gas jet, a separation of solvent molecules and analytes is effected. Removal of solvent vapour is done by a mechanical pump. After repeating this process once more, the resulting particle beam is directed into the ion source. After "flash" vaporization, the analytes can be ionized by EI or chemical ionization, with subsequent mass analysis.

The flow rates that can be accommodated by the PB interface are between 0.1 and 1.0 ml/min, i.e. they are compatible with conventional-size LC. The limiting values are the result of, on the one hand, the minimal flow rate required to form a stable liquid jet and and, on the other hand, the limited capacity to desolvate the aerosol in the desolvation chamber. LC-PB-MS allows one to obtain a lot of relevant structural information from the



PARTICLE BEAM LC/MS INTERFACE

A. WATERS 600-MS LC

B. NEBULIZER

C. DESOLVATION CHAMBER

D. NOZZLE

E. 2 STAGE MOMENTUM SEPARATOR

F. SKIMMER 1

G. 760 L/MIN ROUGH PUMP

WITH BACKSTREAMING FILTER

H. SKIMMER 2

I. 400 L/MIN ROUGH PUMP

WITH BACKSTREAMING FILTER

J. PROBE INLET

K. H-P 5985B EI/CI ION SOURCE

L. HEWLETT-PACKARD 5985B GC/MS

M. HELIUM INLET FOR NEBULIZER

Fig. 15.3. Schematic diagram of a particle beam interface.

generated EI spectra. In addition, the technique is well suited for thermolabile compounds because a limited amount of heat is applied in the desolvation step. This implies that analysis of compounds with low volatility may pose some difficulties. To overcome this problem, in some applications the momentum separator of the PB interface is combined with a thermospray vaporizer [17]. Another interface which uses the TSP principle for vaporization of the LC effluent in a PB set-up, also developed by Vestal and co-workers [18], uses a counterflow gas and a permeable membrane in the desolvation step.

A major drawback of the PB interface is the non-linearity of the signal, which is often observed at low analyte concentrations, and was first reported by Bellar and co-workers [19]. This phenomenon, which is referred to as the "carrier effect", has been the subject of detailed studies [20,21], but the mechanism has not yet been elucidated. To obtain a linear response at low analyte concentrations, compounds such as malic acid [22] or ammonium acetate [23] are added to the mobile phase for either compound-specific or non-specific enhancement of analyte transfer through the interface.

15.2.4. Atmospheric pressure ionization

A new perspective in LC-MS coupling is offered by atmospheric pressure ionization (API) techniques [24,25]. In an API-MS system (Fig. 15.4), the ion source region is separated from the high-vacuum mass analyzer region by a small ion-sampling orifice, which must be large enough to permit the introduction of a large proportion of the ions in the atmospheric pressure region into the vacuum region while maintaining the low pressure required for mass analysis. The LC effluent is sprayed in the vicinity of the orifice, through which ions are sampled into the vacuum region, where free-jet expansion and adiabatic cooling occur. This contributes to the formation of cluster ions, consisting of protonated water or solvent ions attached to the analytes of interest. Dissociation of cluster ions is carried out by directing the ions through an accelerating region or a dry gas curtain. Ionization takes place at atmospheric pressure by either electrospray, ion-spray or atmospheric pressure chemical ionization (APCI). So far, for the determination of pesticides, only ion-spray and APCI applications have been described, probably because of the flow rate limitations imposed on LC associated with electrospray (typically in the 1–5 $\mu\text{l}/\text{min}$ range).

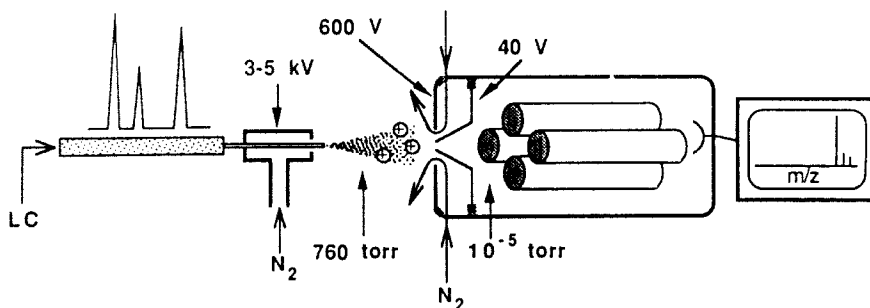


Fig. 15.4. Schematic diagram of an atmospheric pressure ionization interface.

The formation of droplets, which is necessary to create circumstances under which ionization can be carried out, can be effected by using heat, pneumatic nebulization, applying an electrostatic potential or a combination of these. In the ion-spray interface, pneumatic nebulization of the LC effluent exiting from a capillary that is kept at a certain voltage, e.g. 5 kV, results in the formation of charged droplets. Flow rates of 40–50 $\mu\text{l}/\text{min}$, which are compatible with 1 mm i.d. LC columns, can be accommodated. For APCI, heat and pneumatics are combined to volatilize the sample and produce an effluent spray. With this technique, reversed-phase LC effluent flows of 0.1–2.0 ml/min can be handled. Ion formation can be effected by, e.g. corona discharge ionization, which is commonly used with heated pneumatic nebulization and occurs through an electrical discharge at the tip of a needle held at a high voltage (3–6 kV). Another process that can take place is ion evaporation, which results in the emission of ions in solution from charged liquid droplets directly into the gas phase. It should be noted that both APCI and ion evaporation ionization are very mild ionization techniques which readily provide molecular weight information. To obtain useful structural information, it is necessary to carry out collision induced dissociation (CID) experiments. One way of gaining CID information from single analyzer mass spectrometers is to apply accelerating voltages in the free-jet expansion region. With the information so obtained, it may be possible to use API-MS for identification purposes.

15.3. APPLICATIONS

15.3.1. Helium jet direct liquid introduction interface

Micro-LC-MS using the helium jet DLI interface has been used for the analysis of several classes of pesticides. The first application dealt with the determination of 15 phenylurea herbicides [13], separated by isocratic reversed-phase LC. Although not all of the herbicides were chromatographically resolved, MS detection in the PI mode allowed the identification of all compounds, with minimum detectable amounts as low as 100 pg. However, because of the small injection volumes inherent to micro-LC, detection limits were in the ppm range. To overcome the problems of sensitivity, attention was focused on the development of miniaturized preconcentration equipment in order to allow the analysis of larger sample volumes of correspondingly lower concentrations. Preconcentration involved the use of a 5×2 mm i.d. precolumn, packed with the same material as the analytical column [26]. Preconcentration of a 10-ml sample containing several phenylurea herbicides resulted in detection limits of 10 ppb in the PI mode when using selected ion monitoring (SIM). The trace enrichment procedure caused some additional band broadening, but the loss of resolution was easily made up for by the selectivity of the MS.

In another study Apffel et al. used an on-line extraction system suitable for DLI to allow the addition of non-volatile mobile phase modifiers, and to introduce postcolumn derivatization techniques for enhanced selectivity and sensitivity [27]. In the postcolumn extraction system, 1,2-dichloroethane was added to the aqueous LC effluent by a syringe pump, with extraction taking place in a 300×0.3 mm i.d. PTFE extraction coil. Subsequently, the aqueous and organic phases were separated using a phase separator

containing a membrane permeable only to non-polar organic solvents. The phenoxy acid herbicides 2,4-D, 2,4,5-T and silvex were used as model compounds. Because of the electronegativity of the chlorine atoms in the chlorinated acids, relatively strong negative ion signals, compared with positive ion signals, were obtained. The examples described demonstrate the feasibility of incorporating an extraction system into an LC-MS set-up. No detection limits were reported.

The addition of a halogenated modifier to the LC eluent, with the aim of improved detection performance in NCI-MS, was investigated for various chlorophenols, 2,4-D and 2,4,5-T [28]. The addition of 0.1–1% chloroacetonitrile, resulting in chloride enhanced negative ionization (CLENI), had a profound effect on the mass spectra of all chlorophenols tested. With phenol and the low-chlorinated phenols, the $[M + Cl]^-$ cluster became the dominant peak (instead of $[M - 1]^-$), while the detectability was improved considerably. With the tri- and higher chlorinated phenols, $[M - 1]^-$ invariably became the base peak (instead of $[M - Cl]^-$); here the detection sensitivity increased only modestly, if at all. Similar behaviour was observed for the phenoxy acids, with chloride attachment occurring more easily with 2,4-D. Halogenated modifiers such as trifluoroacetic acid, trifluoroethanol and bromoacetonitrile proved not to be suitable additives for various reasons. Monitoring of the reagent gas ion spectra of fluorine-containing additives revealed that no $[F]^-$ was formed, whereas a disadvantage of the use of bromoacetonitrile was the relatively high mass of bromine, which poses limitations on the scan range. Analyte detectability was not studied extensively; the authors report that 3 ng of pentachlorophenol could be detected in the NI mode, which implies a detection limit of 6 ppm with an injection volume of 0.5 μ l.

Application of CLENI was also studied for a number of organophosphorus pesticides, and compared with positive and negative chemical ionization (PCI and NCI, respectively) [29]. NCI and PCI generally provided information about the functional group of a pesticide and its molecular weight, respectively. In CLENI, an intense $[M - R]^-$ (R = methyl, ethyl) was always formed as well as, in most instances, a relatively intense functional group fragment. For the trace-level determination of the organophosphorus pesticides, NCI and CLENI are to be preferred to PCI because of their higher signal intensities. This is illustrated in Fig. 15.5, which shows the total ion current (TIC) chromatogram of a mixture of the ten organophosphorus pesticides in the PCI, NCI and CLENI mode. With SIM, detection of around 50 pg was obtained in the NI mode, indicating detection limits of 100 ppb with an injection volume of 0.5 μ l. LC-NCI-MS and LC-CLENI-MS were used to determine paraoxon-methyl, azinphos-methyl and ronnel in spiked soil samples. The results compared favourably with those of LC-UV analysis.

The above examples clearly show that trace enrichment, preferably through the use of on-line precolumn techniques, is a necessary step in method development for environmental analysis. Only then can the requirements for high sensitivity associated with this type of analysis be met and low-ppb detection limits be obtained [26].

Despite the satisfactory results obtained with micro-LC helium jet DLI-MS, no applications of this interface for the determination of pesticides have been reported since 1989. No doubt this is mainly due to the fact that only low LC eluent flow rates can be tolerated and, probably more importantly, that really polar analytes – which are of most interest here – cannot be handled with this interface.

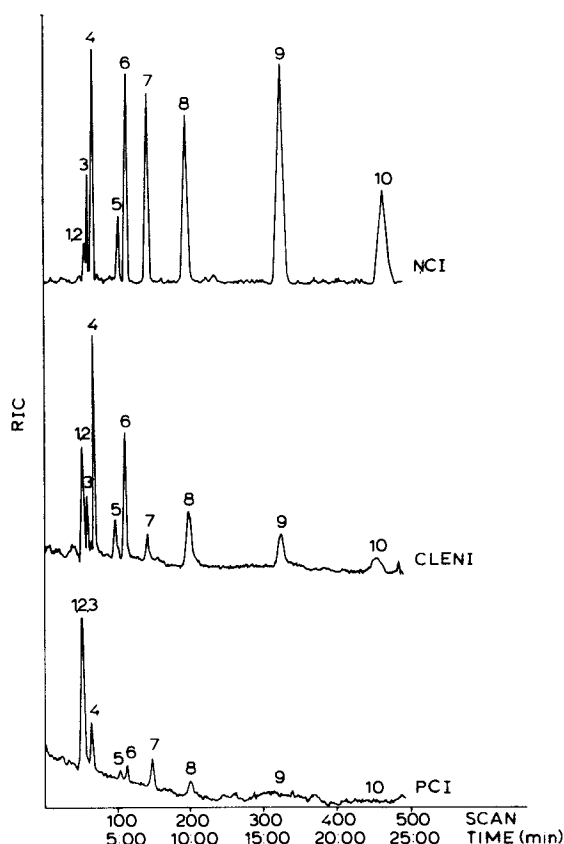


Fig. 15.5. Reconstructed ion current chromatograms in LC-PCI-MS, LC-NCI-MS and LC-CLENI-MS for a mixture of (1) vamidothion, (2) trichlorfon, (3) dimethoate, (4) paraoxon-methyl, (5) azinphos-methyl, (6) parathion-methyl, (7) azinphos-ethyl, (8) coumaphos, (9) ronnel and (10) carbophenothion. Amount of each component injected, 50 ng; eluent, acetonitrile-water (70:30) for PCI and NCI or acetonitrile-water-chloroacetonitrile (69:30:1) for CLENI at 20 μ l/min; scan time, 3 s; ion source temperature, 300°C [29].

15.3.2. Thermospray interface

After the initial successful use of the TSP interface, much research was directed towards enhancing selectivity and creating the possibility of identifying unknown compounds. To this end, the addition of mobile phase constituents such as inorganic salts and halogenated organic modifiers and the use of normal-phase LC and postcolumn extraction were studied.

The applicability of LC-TSP-MS in environmental analysis is demonstrated by the wide range of pesticides that has been analyzed using this technique. The work of, e.g.

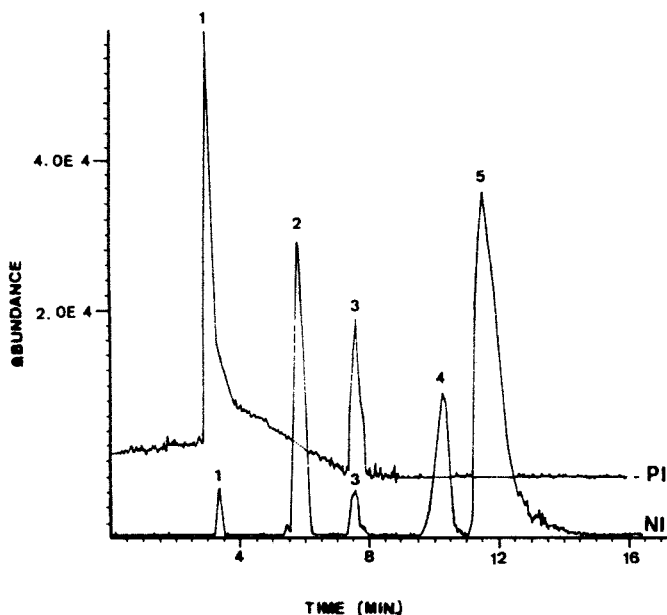


Fig. 15.6. Reconstructed ion chromatograms in TSP, LC-PI MS (PI) and TSP, LC-NI MS (NI) for mixture: 1, cyanazine; 2, 2,4-dichlorophenol; 3, linuron; 4, 2,4,5-trichlorophenol; 5, pentachlorophenol. Injected amount of each component, 100 ng (except compound 5, 300 ng). Carrier stream: methanol/water (70:30) + 0.1 M ammonium acetate + 0.2% acetic acid. Flow rate: 1.2 ml/min [34].

Barceló et al. covers organophosphorus insecticides, chlorophenols, triazines, phenylurea herbicides, phenoxy acids and carbamates.

The characterization of organophosphorus compounds such as trichlorfon, vamidothion [30], ronnel, azinphos ethyl [31], diazinon, parathion methyl [32] and dimethoate [33], was achieved by utilizing PI and NI filament-on TSP-MS in combination with reversed-phase LC. Using ammonium acetate as additive, the positive ions formed are $[M + H]^+$ and $[M + NH_4]^+$, depending on the proton affinity of the compound under investigation relative to that of ammonia. In the NI mode, three mechanisms of ion formation are distinguished: (i) anion attachment, resulting in the formation of $[M + MeCO_2]^-$; (ii) electron capture, with $[M]^-$ as the product; (iii) dissociative electron capture, leading to the formation of fragment ions through the loss of a functional group. Several chlorophenols, the phenylurea herbicide linuron and the triazine cyanazine have been analyzed in the same way [34], with the chlorophenols showing no response in the PI mode (see Fig. 15.6). In general, signal intensities in the PI mode were about three orders of magnitude higher than in the NI mode, except for linuron and cyanazine, where sensitivities differed only by one order of magnitude; lowest detectable amounts ranged from 5 to 50 ng.

Several *N*-methylcarbamates, i.e. aldicarb, carbofuran and carbaryl, and the phenylureas, linuron and diuron, have been analyzed by Voyksner et al. using gradient elution [35]. Ammonium acetate was added postcolumn to prevent deterioration of the LC separation. Lowest detectable amounts varied from 1 ng for carbofuran to 80 ng for diuron in the PI mode; in the NI mode the sensitivity was 4–5 orders of magnitude lower.

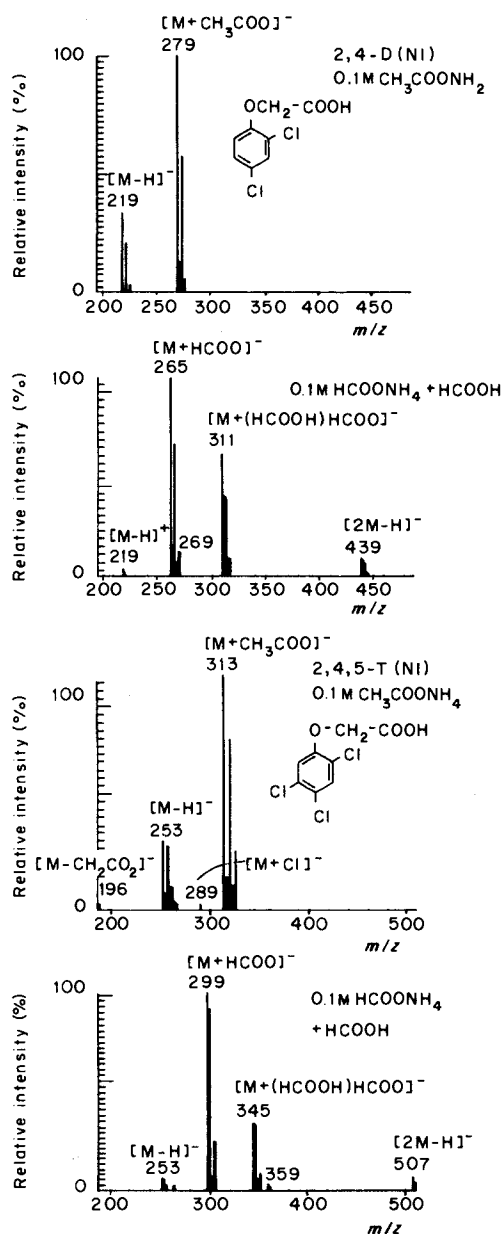


Fig. 15.7. Direct flow injection TSP NI MS of 2,4-D and 2,4,5-T. Carrier stream: methanol/water (50:50) + 0.1 M ammonium acetate or 0.1 M ammonium formate. Flow rate: 1.2 ml/min. Injection: 300 ng [40].

For sulfonylureas, a loss in TSP signal was observed using a mobile phase acidified with 0.1 M acetic acid rather than a neutral mobile phase [36]. To overcome this problem, ammonium hydroxide was added postcolumn to neutralize the acid required during separation. The neutralization process also supplies the ammonium acetate needed for TSP ionization. The sulfonylureas present as the active ingredients in the herbicides Harmony, Glean and Londax have been analyzed in crops at the 50 ppb level, using SIM for quantitation [37].

Voyksner [38] described the determination of carbamates, phenoxy acids, chlorinated aliphatic acids, phenylurea herbicides and oxime fungicides in environmental samples spiked at the 1 ppb level. Compounds were separated by gradient elution, with ammonium acetate in the mobile phase. Sample pretreatment was carried out by means of liquid-liquid or liquid-solid extraction for soil and water samples, respectively. Under full scan conditions, the various compounds could be detected at the 0.1–1 ppb level, with estimated detection limits in the 10 ppt range. Chlorinated pesticides, such as linuron, were analyzed in the NI mode.

Besides ammonium acetate, several authors have used ammonium formate as an additive in order to obtain structural information and/or to increase the scan range at the low mass end. A study dealing with the determination of organophosphorus insecticides and triazines and the applicability of triethylamine and ammonium carbonate, bicarbonate, formate and acetate as additives in LC-TSP-MS was carried out by Voyksner et al. [39]. Ammonium acetate gave the highest sensitivities, with detection levels of 20–60 ng for the triazines and 30–300 ng for the organophosphorus insecticides; however, ammonium formate is also regularly used.

When determining triazines, phenylureas, chlorinated phenoxy acids [40] and carbamates [41], the adduct ions formed with ammonium formate provide complementary structural information which enables unambiguous molecular weight assignment for unknown pesticides. This is demonstrated in Fig. 15.7, which shows mass spectra obtained after flow injection analysis of 2,4-D and 2,4,5-T in the NI mode with ammonium acetate and ammonium formate. Detection limits for the triazines simazine, atrazine and propazine were 5 ppb, using SIM, while carbamates could be detected at the 50 ppb level, under full-scan conditions.

In another effort to obtain additional structural information, normal-phase LC eluents were used, as is illustrated by the determination of phenoxy acids and chlorotriazines [42]. With cyclohexane as the mobile phase (no ammonium acetate added), the phenoxy acids 2,4-D, 2,4,5-T and silvex showed good sensitivity in the PI mode in LC-TSP-MS. Non-polar solvents were also used to determine a number of organophosphorus insecticides and chlorophenols [43]. In this study, the influence of *n*-hexane, cyclohexane and dichloromethane – which are well-known constituents of normal-phase LC eluents – on the chemical ionization process during the generation of TSP mass spectra was evaluated. In the PI mode, the detection limits improved about 10-fold for all test compounds when using a normal-phase instead of a reversed-phase eluent; organophosphorus insecticides such as trichlorfon, fonofos and parathion-ethyl, could be detected at the 1–10 ng level. In the NI mode, the chlorophenols, 2,4-dichlorophenol, 2,4,5-trichlorophenol and pentachlorophenol, showed the same sensitivity with both normal- and reversed-phase eluents, with detection levels ranging from 1 to 10 ng.

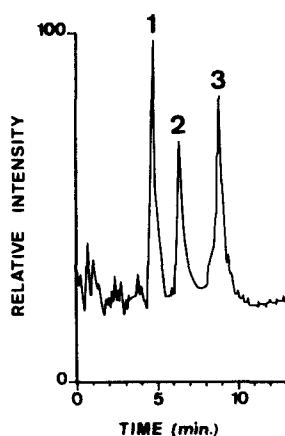


Fig. 15.8. Reconstructed ion chromatogram obtained with NI TSP LC-MS of a water sample from the Barcelona harbour spiked with 0.1 ppm of (1) 2,4-D, (2) 2,4,5-T and (3) silvex. Stationary phase, 5 μ m LiChrosorb RP-18; mobile phase, acetonitrile/water (50:50) + 0.1 M phosphate buffer (pH = 2.5); flow rate, 1 ml/min; extraction solvent, cyclohexane/dichloromethane/1-butanol (45:45:10) at 1 ml/min; flow rate into MS, 0.8 ml/min [43].

An interesting aspect of the suitability of non-polar solvents for LC-TSP-MS is the possibility of postcolumn extraction, enabling the use of aqueous eluents containing non-volatile constituents such as phosphate buffers for the LC separation. After ion-suppressed reversed-phase LC of 2,4-D, 2,4,5-T and silvex [43] using a phosphate buffer, the analytes were extracted on-line into a non-polar solvent mixture and, after phase separation in a sandwich phase separator, introduced into the LC-TSP-MS system. Figure 15.8 shows the chromatogram (full scan, NI mode), obtained for a water sample from the Barcelona harbour spiked with 0.1 ppm of each phenoxy acid, obtained after reversed-phase LC combined with postcolumn extraction. Introduction of an extraction step into the analytical set-up can also be effected by integrating a segmented-flow system, as described by Farran et al. [44] for the determination of organophosphorus insecticides, chlorophenols and phenoxy acids. The analytes are extracted from the aqueous sample into *n*-heptane and, after phase separation, the organic phase is led through the sample loop of the injection valve of the LC system.

Since chemical ionization occurs in the TSP interface, adduct formation is strongly dependent on the LC eluent composition, as was demonstrated by using different additives [40,41] and non-polar solvents [42,43]. In order to obtain additional structural information, the use of chloroacetonitrile as eluent additive has been studied. The utility of solvent adduct ions was shown for the determination of phenoxy acid herbicides in the NI mode [45], where addition of 2% chloroacetonitrile to reversed-phase eluents caused the $[M + Cl]^-$ ion to be the base peak, thereby demonstrating abundant chloride attachment. The same behaviour was observed for various chlorophenols in a more detailed study on adduct formation in the filament-off, filament-on and discharge modes [46]. An interesting aspect is the difference in mass spectral information obtained when using a Hewlett Packard or a Finnigan MAT interface. Figure 15.9 shows that with the Hewlett

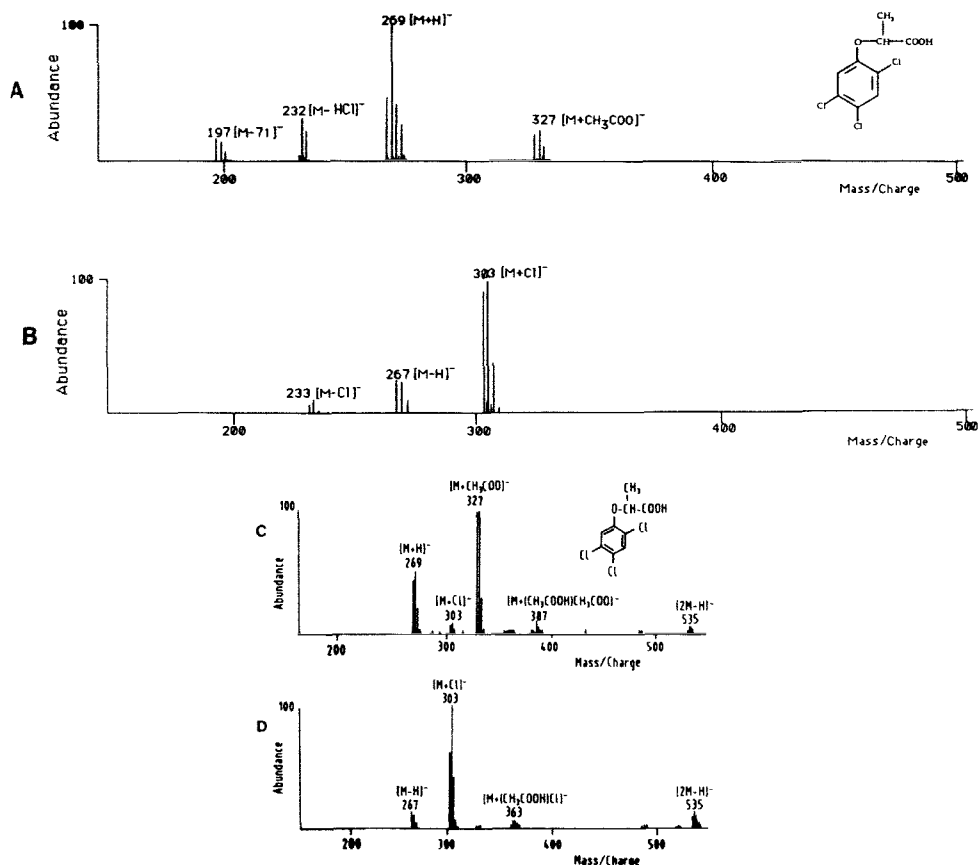


Fig. 15.9. FIA TSP NI mass spectra of silvex with the Finnigan MAT (a,b) and Hewlett Packard (c,d) interfacing systems; filament-on operation. Carrier stream: (a,c) water/acetonitrile (50:50) + 50 mM ammonium acetate and (b,d) water/acetonitrile/chloroacetonitrile (49:49:2) + 50 mM ammonium acetate. Flow, 1 ml/min; injected amount, 5 μ g [46].

Packard interface there is a higher tendency to form acetate adduct ions in the absence of chloroacetonitrile than with the Finnigan MAT interface, which is better suited for electron-capture processes. Upon addition of 2% chloroacetonitrile to the mobile phase, $[M + Cl]^-$ becomes the base peak for both interfaces. Using a mobile phase containing 2% chloroacetonitrile, detection limits for 2,4-dichlorophenol, 2,4,5-trichlorophenol and pentachlorophenol in the filament-on mode were in the low-nanogram range.

The different approaches to obtain structural information from TSP mass spectra described above – use of ammonium formate as well as ammonium acetate, use of non-polar solvents, addition of chloroacetonitrile to the mobile phase – are summarized in ref. 47 for a variety of carbamates, chlorinated phenoxy acids, chlorotriazines, organophosphorus insecticides and phenylurea herbicides. In addition, postcolumn extraction (cf. [43]) was combined with ion-pair reversed-phase LC for difenzoquat, a quaternary ammonium pes-

ticide, which could be extracted into an organic solvent as an ion-pair with a sulphonate-type counter ion. Although the results are encouraging, there is still a need to improve the analytical system because the volatility of the ion-pairing agent is a critical parameter.

Considering the results obtained with LC-TSP-MS in environmental analysis, it cannot be denied that despite the various efforts to derive structural information from the mass spectra, identification of unknown compounds is still a major point of concern. Although several authors state that the use of different mobile phase additives allows one to obtain structural information, a critical comment seems appropriate. Obviously, different ions show up in the mass spectra, but the information made available can in most cases only be used to establish the molecular weight of the analyte in a different way. In other words, no substantially new information is provided.

The merits and drawbacks of LC-TSP-MS for environmental analysis are discussed in several excellent studies, in which techniques essential for routine trace analysis such as preconcentration and clean-up are integrated in the total analytical approach. Bellar and Budde explored the possibility of developing a broad-spectrum method for the determination of non-volatile target compounds in aqueous environmental samples, using off-line extraction and concentration techniques [48]. LC separation of samples containing a wide range of pesticides such as carbamates, sulfonylureas, triazines, phenylureas and organophosphorus compounds, spiked at the 2–50 and 20–500 ppb level for liquid-liquid and liquid-solid extraction, respectively, was effected by gradient elution. Co-elution of a few analytes was acceptable because the probability of all analytes being present in a single sample is very low. Besides, when two compounds co-elute, MS detection using different characteristic ions will generally allow their recognition and separate determination. Figure 15.10 shows the TIC chromatogram of a mixture of 34 compounds of environmental interest, obtained by filament-off ionization and detection in the PI mode. With the

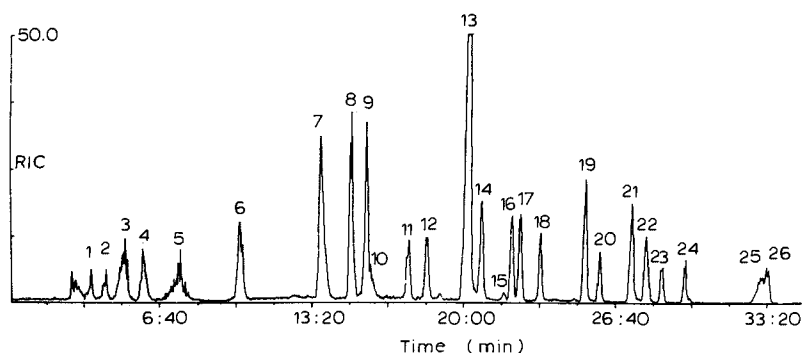


Fig. 15.10. Total ion current profile from the LC-filament-off TSP-MS determination of a mixture of 34 compounds of environmental interest (adapted from [48]). Assignment of selected peaks: 1, aldicarb sulfoxide; 2, caffeine; 3, aldicarb sulfone + oxamyl; 4, methomyl; 5, coumafuryl; 6, atrazine, dealkylated; 7, metribuzin, deaminated; 8, *N*-(1-naphthyl)thiourea; 9, fenamiphos sulfoxide + warfarin; 10, carboxin sulfone + aldicarb; 11, monuron; 12, cyanazine; 13, D_6 and D_4 dimethyl phthalate + propoxur; 14, carbofuran; 15, D_5 atrazine; 16, fluometuron; 17, thiofanox + carbaryl; 18, diuron; 19, propachlor; 20, propham; 21, siduron; 22, BPMC + methiocarb; 23, linuron; 24, mexacarbate; 25, alachlor; 26, rotenone.

TABLE 15.1

CALIBRATION, RESPONSE, AND TOTAL METHOD ACCURACY AND PRECISION DATA FOR 29 ANALYTES OBTAINED WITH LIQUID-LIQUID EXTRACTION AND FILAMENT-OFF LC-TSP-MS [48]^a

Compound	Calibration data			Liquid-liquid extraction		
	Amount injected (ng)	Mean response ^b (area/ng)	RSD	Water concn. (µg/l)	Mean % accuracy (RSD)	Method detection limit ^c (µg/l)
Alachlor (Lasso)	400	107	12	20	78 (9.5)	5.0
Aldicarb (Temik)	100	149	5.0	5	66 (15)	1.6
Aldicarb sulfoxide	300	13	23	15	19 (18)	1.8
Aldicarb sulfone	300	38	8.8	15	95 (17)	8.0
Atrazine, dealkylated	72	1270	8.9	3.6	73 (12)	1.0
BPMC (Osba)	100	80	7.9	5	59 (21)	2.1
Carbaryl (Sevin)	980	14	6.5	49	88 (11)	16
Carbofuran	200	211	10	10	83 (9.0)	2.5
Carboxin sulfone	260	67	18	13	99 (9.2)	4.0
Coumafuryl	200	77	11	10	17 (12)	0.7
Cyanazine (Bladex)	50	340	22	2.5	76 (5.0)	0.2
Diuron	100	165	18	5	73 (5.7)	0.7
Fenamiphos sulfoxide	180	196	26	9	75 (4.5)	1.1
Fluometuron	100	537	14	5	85 (5.7)	0.8
Linuron (Lorox)	1000	15	15	50	85 (12)	18
Methiocarb	70	703	10	3.5	93 (3.3)	0.4
Methomyl (Lannate)	40	1170	18	2	68 (12)	0.6
Metribuzin, deaminated	100	965	9.4	5	51 (3.1)	0.3
Mexacarbate (Zectran)	100	508	12	5	85 (7.5)	1.1
Monuron	50	569	14	2.5	78 (7.0)	0.5
N-(1-Naphthyl)thiourea	200	138	19	10	39 (17)	2.2
Oxamyl	100	22	30	5	69 (11)	1.3
Pronamide hydrate	140	132	15	7	86 (7.8)	1.6
Propachlor (Ramrod)	100	747	9.5	5	65 (16)	1.7
Propham	1000	8	8.5	50	52 (16)	14
Propoxur	600	19	11	30	69 (16)	11
Rotenone	400	33	13	20	66 (13)	5.8
Siduron	100	470	14	5	84 (6.5)	0.9
Warfarin	200	106	15	10	74 (10)	2.5
Grand mean	260	306	14	13	71 (11)	3.7

^aData from five calibrations and six extractions all measured within a 9-h period on the same day.

^bQuantitation ion abundances integrated over the LC peak. ^cMinimum concentration that can be measured with 99% confidence that concentration is greater than zero.

liquid-liquid preconcentration procedure, detection limits for the analytes varied from 0.2 ppb for cyanazine to 16 ppb for carbaryl (Table 15.1). Detection limits obtained via liquid-solid extraction are approx. 10 times higher, mainly because of the small size and limited capacity of the commercially available extraction cartridges used in this study. Combination of retention time data, molecular weight information from the $[M + H]^+$ and $[M + NH_4]^+$ ions, evidence from ions containing naturally occurring isotopes, and occasional fragment ions, provides satisfactory information for the identification of target analytes without a large risk of false positives.

As an extension of the work of Bellar and Budde to the analysis of fruits and vegetables, some 20 carbamates and phenylureas have been determined in crops via a preconcentration procedure based on off-line extraction [49,50]. In general, detection limits obtained by SIM in the PI mode were found to range from 0.25 ppm for the phenylurea compounds to 1 ppm for the carbamates. For compounds such as fenvalerate and folpet, NI detection resulted in detection limits of 0.025–0.1 ppm. All detection limits were lower than or equal to the tolerances set by the United States Environmental Protection Agency (US EPA), except for chlorbromuron, fenvalerate, metobromuron and oxamyl in certain crops.

A further illustration of the possibility of identifying target compounds is the analysis of river-water samples [51]. Concentration of 1 l of river-water spiked at the 5 ppb level to 1 ml by means of solid-phase extraction, followed by desorption into methanol with subsequent injection of 20 μ l onto the analytical column enables the detection of several triazine and phenylurea pesticides at the low-ppb level under full-scan conditions. As an example, a dichloromethane extract of a riverwater sample known to be contaminated with isoproturon was analyzed to provide confirmation of pollutant identity. A major peak exhibiting an ion at m/z 207 had the same retention time as an isoproturon standard and gave a similar mass spectrum, thereby demonstrating the confirmatory power of LC-TSP-MS.

The feasibility of incorporating sample clean-up and trace enrichment into the total analytical set-up, providing an easily automatable system for routine monitoring, is described by Bagheri et al. [52] for the determination of a number of phenylurea herbicides in surface and drinking water. Sample pretreatment was carried out by using either a 10×2 mm i.d. stainless-steel precolumn packed with 15–25 μ m PLRP-S styrene-divinylbenzene copolymer or a home-made membrane disk holder filled with 4.6 mm diameter C-18-bonded silica membrane extraction disks. After trapping the analytes from a 50-ml sample on the precolumn or extraction disks, desorption in the backflush mode was effected with methanol/0.1 M ammonium acetate (40:60, v/v) and analytes were transferred on-line to a C-18 analytical column. The actual separation of the analytes was carried out using a linear gradient. In the thermospray interface, ions were generated under discharge conditions and data were acquired in the PI mode. All phenylureas used in this study generated $[M + H]^+$ as the base peak, with $[M + NH_4]^+$ observed as an adduct ion in some cases. In order to improve the selectivity and sensitivity of the system as much as possible, the chromatograms were recorded under time-scheduled SIM conditions. In river Rhine water samples spiked with a mixture of 15 phenylurea herbicides at levels ranging from 0.05 to 10 ppb, detection limits for all compounds except linuron and chlorobromuron were found to be 5–15 ppt. For linuron and chlorobromuron the limits of detec-

tion were 60 and 120 ppt, respectively. With this system, the presence of two phenylurea herbicides, monuron and isoproturon, at very low levels in river Rhine water could be confirmed. As demonstrated, on-line preconcentration in combination with reversed-phase LC-TSP-MS is a fast, sensitive and selective method for the identification and determination of phenylurea herbicides.

15.3.4. Particle beam interface

The use of the PB interface for the LC-MS determination of pesticides, with the possibility of obtaining structural information from EI ionization mass spectra, was first described in 1990 [53]. In a sequel to their earlier work with the TSP interface, Behymer et al. described the performance of the PB interface in the LC-MS analysis of a number of carbamates and phenylurea herbicides. A reversed-phase gradient separation was developed and ammonium acetate added to the mobile phase because of the beneficial effect on the transfer efficiency of the analytes in the interface. It should be noted that the nature of this effect still has not been elucidated [20,21]. Mass spectra obtained during this work were classical EI spectra, without any evidence of adduct ions formed with ammonium acetate. The detection limits for the various analytes ranged from 10 to 440 ng.

LC-PB-MS has been used as a confirmatory tool for the determination of several chlorinated phenoxy acids in spiked soil samples [54,55]. Efforts to use the technique for quantitation were not successful because the instrument response factors were found to vary greatly in a limited period of time. For analyte confirmation, the mass spectra obtained with EI ionization were compared with the corresponding NBS-Wiley reference library mass spectra (Hewlett Packard) [54]. The reference spectra are in a condensed form and do not show every ion present in the original spectra, but comparison shows that they are visually very similar. The same ions are present in the reference and the particle beam spectra, although their relative intensities are somewhat different.

The highly polar plant growth regulator daminozide has been analyzed by anion-exchange LC-PB-MS [22,56]. The presence of malic acid in the LC eluent, in concentrations ranging from 0 to 4 mM yields a 30-fold signal enhancement in the PCI mode (with isobutane as reagent gas); in addition, the response becomes linear. With 4 mM malic acid in the LC eluent, a detection limit of 25 ppb is obtained.

The use of mobile phase additives to "carry" the analyte through the PB interface in order to improve detection limits and linearity of the analyte response was also reported for several chlorinated phenoxy acids [57] and phenylurea herbicides [58]. In both instances, signal enhancement is effected by adding a structurally similar carrier – phenoxy acetic acid and phenylurea, respectively – in low-ppm concentrations to the mobile phase. Methane NCI combined with SIM resulted in detection limits of 1.1 ppb for 2,4-D, 2,4,5-T and silvex, and of 0.16 and 0.5 ppb for diuron and linuron, respectively.

Ethylene-thiourea, a notorious degradation product of the fungicide ethylene-bis(dithiocarbamate), has been determined in crop extracts in the presence of [^{13}C]ethylene-thiourea, which was used as an internal standard [59]. Detection limits were 5 ppb under full-scan conditions. Flow injection analyses showed that co-elution of [^{12}C]ethylene-thiourea and the ^{13}C -labelled compound did not affect the molecular ion intensity. These results are in contrast with previous studies on the use of several commer-

cial PB interfaces; here a ca. twofold signal enhancement was found for co-eluting isotope-labelled compounds [19].

A study on over 100 analytes from the US EPA National Pesticide Survey (NPS), demonstrated the practicability of LC-PB-MS for the identification and quantitation of non-volatile polar pesticide residues in groundwater [60]. Because response curve shapes (either linear or exponential) have a physical basis and a compound-specific mass transport efficiency effect exists that can have a linear or exponential dependence on the total amount of analyte transported through the PB interface, no carrier was added to the mobile phase. Estimated detection limits for selected pesticides ranged from 5 ng for carboxin sulfoxide to 50 ng for disulfoton sulfoxide. The authors concluded that, since LC-PB-MS is not sufficiently sensitive for the determination of all 104 NPS compounds, it may be necessary to combine the method with, e.g. TSP, electrospray or other API MS techniques, for complete confirmation of all contaminants.

In an interlaboratory evaluation of LC-MS for the analysis of ten chlorinated phenoxy acid herbicides, the performance of the PB and the TSP interface was compared [61]. The authors found that the choice of the interface will depend on the type of analytes studied and the analytical requirements. They conclude that for the determination of low levels of phenoxy acids, NI-TSP-MS is preferred. When, at the higher analyte levels, identification is essential, PB with electron impact ionization might be preferable. It should be pointed out that the problems encountered in this study concerning low-level detection with the PB interface – in this case, 5 µg/ml – are not common practice, as is demonstrated by detection levels of 1 ng in the NI mode obtained for some chlorinated phenoxy acids when using a carrier [57]. This certainly detracts from the value of this interlaboratory comparison.

The suitability of the PB interface for the identification of non-target analytes is illustrated by the analysis of effluents from treatment plants, where LC-PB-MS was used to identify compounds which went undetected in on-column GC-MS [23]. After off-line sample concentration of 10 l of wastewater to 1 ml, an aliquot was injected and gradient

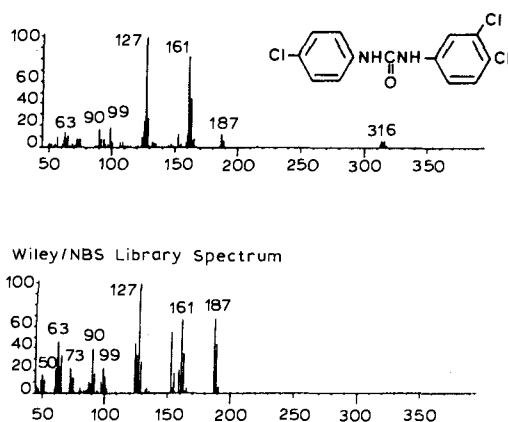


Fig. 15.11. Comparison of spectrum obtained by LC-PB-MS with Wiley/NBS library spectrum of *N*-(4-chlorophenyl)-*N'*-(3,4-dichlorophenyl)-urea [23].

elution carried out with 0.01% ammonium acetate added to the mobile phase as a carrier. In Fig. 15.11, the EI spectrum of triclocarban, a phenylurea present in the sample, is shown together with the reference spectrum from the Wiley/NBS library. The low detection levels for triclocarban, viz. in the low-ppb range, indicate that LC-PB-MS can be a useful tool for non-target analysis, provided a sufficient amount of sample is available.

The possibility of obtaining useful structural information by means of PB(EI)-MS is demonstrated with the analysis of the isobaric triazines terbutylazine and propazine (molecular weight, 229) [62]. Figure 15.12 shows that the TSP mass spectra do not provide any information except for the molecular weight. Using the PB spectra, however, propazine and terbutylazine can be differentiated on the basis of the structurally relevant fragment ions detected. Nevertheless, a well-founded comparison of the potential of the two techniques is not possible on the basis of this study because of the lack of information concerning the sensitivity obtained with the PB interface.

The above examples show that the results obtained with the PB interface in environmental LC-MS analysis differ considerably from one study to another. Consequently, the expectations concerning the role this interface will play in the future vary widely. The performance of the PB interface has been criticized especially in terms of sensitivity, despite the satisfactory detection limits reported in several studies. No conclusion can yet be drawn regarding the value of the PB interface in comparison to the TSP interface, but it should be recognized that with the former interface structural information not obtainable by TSP techniques becomes available.

15.3.5. Atmospheric pressure ionization

So far, the number of published applications of API-MS for the determination of pesticides is very limited. Nevertheless, the expectation that this technique will play an important role in environmental analysis seems justified, as is illustrated by the following examples.

Analysis of a mixture of selected carbamates and phenylurea herbicides was described for both ion-spray and heated pneumatic nebulizer LC-MS [63]. Gradient elution LC was used with ammonium formate as additive to the mobile phase. In the case of ion-spray, the column effluent (0.4 ml/min) was split so that approx. 20 μ l/min were delivered to the ion-spray interface. By applying a potential difference of 20–40 V between the ion-sampling capillary and the skimmer in the first pumping stage, CID of the analytes was obtained, together with dissociation of the solvent cluster ions; this resulted in an improved signal-to-noise ratio. The ion-spray and APCI total ion current responses for the individual components were found to differ. This was attributed to differences in the ionization mechanisms of the two techniques and to the thermolability of the compounds analyzed. As a consequence of the heat applied in the APCI process, the CID APCI mass spectra show more abundant low-mass fragment ions at the expense of the parent ions in comparison with the ion-spray mass spectra. All the CID mass spectra display several fragment ions and are well suited for SIM CID LC-MS experiments, should SIM be needed for the analysis of environmental samples. No detection limits are reported in this study.

Low-nanogram detection limits were obtained by APCI for a number of organophosphorus compounds [64]. The compounds studied were classified into three groups, viz.

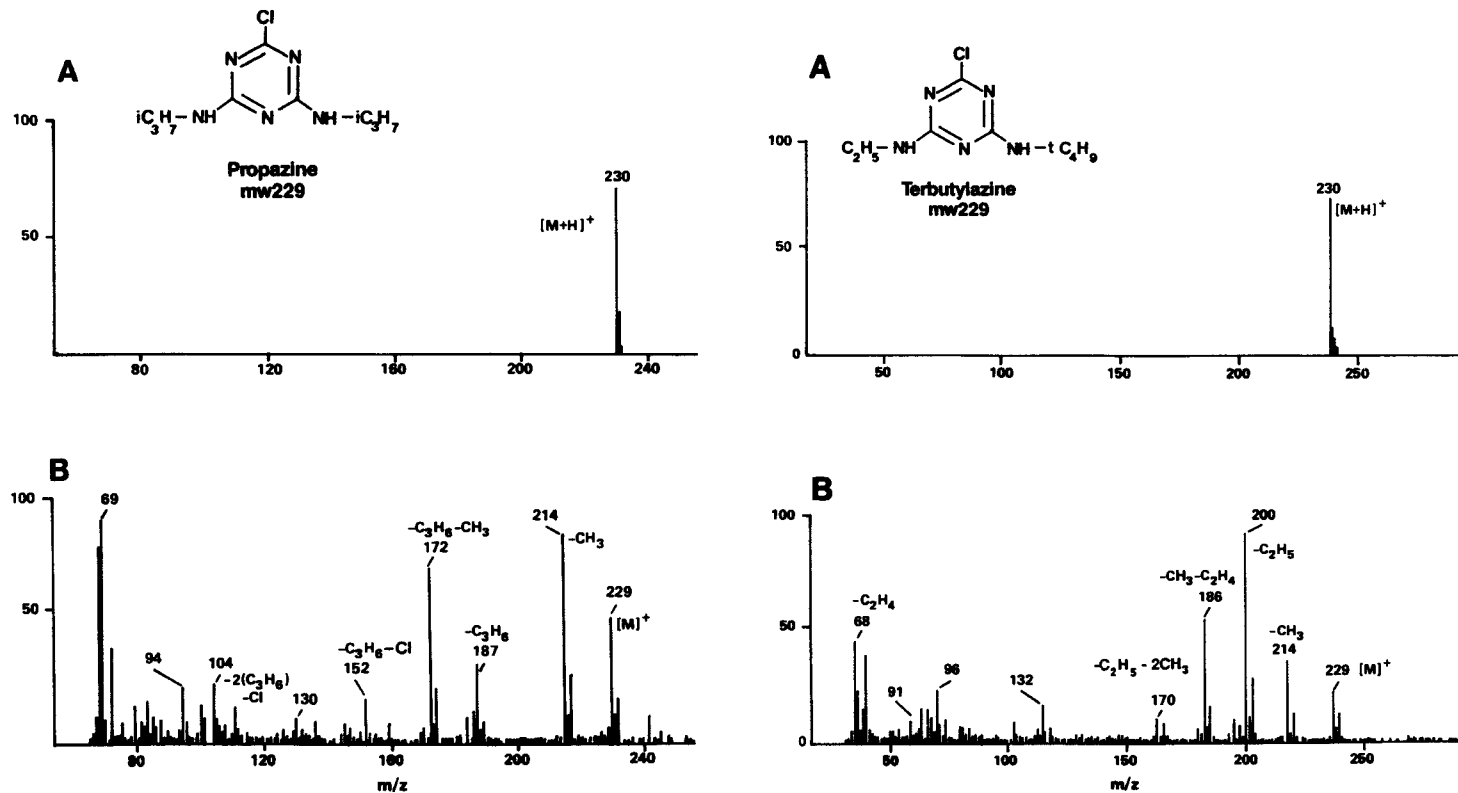


Fig. 15.12. Mass spectra of propazine (left) and terbutylazine (right): (A) thermospray spectra, (B) particle beam EI spectra [62].

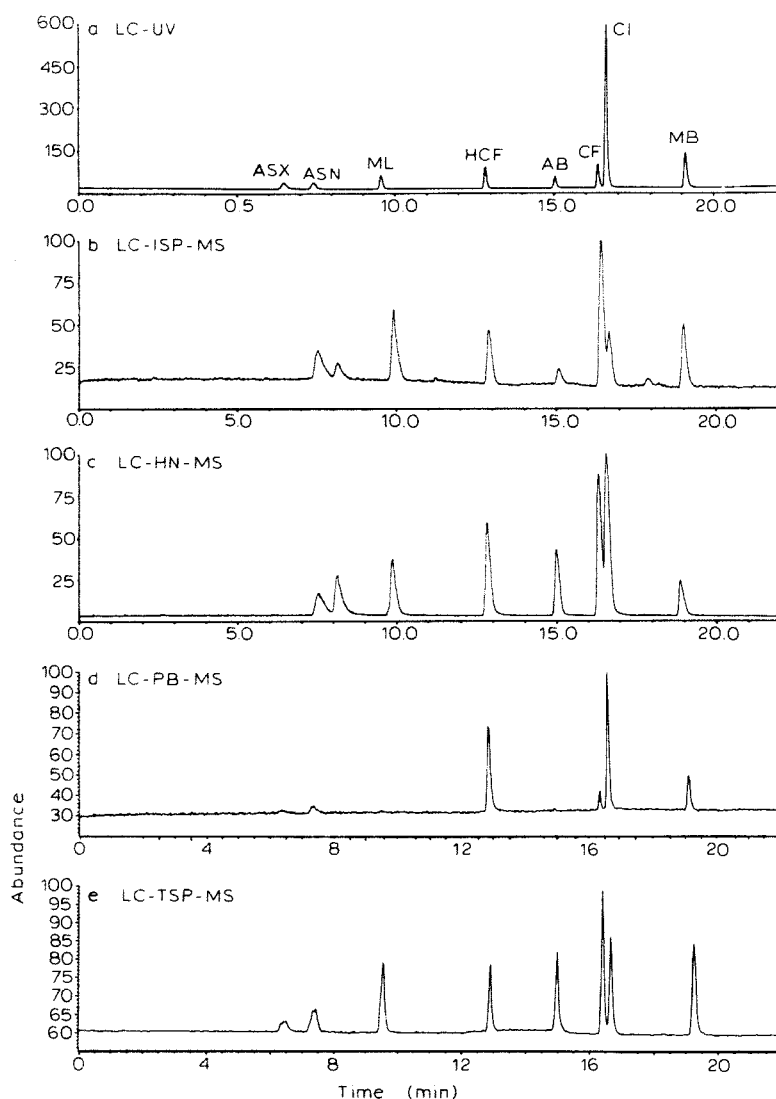


Fig. 15.13. LC separation of eight *N*-methylcarbamate pesticides with detection by (a) UV (214 nm) and mass spectrometry using (b) ion-spray, (c) APCI, (d) PB-EI and (e) TSP interfaces. Conditions: column, 250 × 4.6 mm i.d. Zorbax RX-C8; mobile phase, aqueous methanol for (a) and (c), modified with formic acid for (b), all at a flow rate of 1 ml/min; gradient profile, held at 20% methanol for 4 min, followed by a linear gradient to 70% methanol in 11 min and held for 5 min; split ratios, 1:20 for (b) and 3:2 for (d), postcolumn addition of 0.2 ml/min of 0.5 M ammonium acetate for (e); injection volume, 20 μ l (20 μ g/ml for (a) and (d), and 2 μ g/ml for (b), (c) and (e)). Peaks: ASX, aldicarb sulfoxide; ASN, aldicarb sulfone; ML, methomyl; HCF, 3-hydroxycarbofuran; AB, aldicarb; CF, carbofuran; CI, carbaryl; MB, methiocarb [65].

detectable only by PI measurement, only by NI measurement and by both PI and NI measurement, the classification being dependent on the acidity of the compound in question. It was found that the presence of ammonium acetate, sometimes used as mobile phase additive to enhance the separation efficiency, caused a decrease in detection sensitivity. Detection limits obtained by SIM generally were in the low-nanogram range, i.e. 2 ng for dichlorvos and dimethoate in the PI mode and for parathion and fenitrothion in the NI mode. Significant structural information could be derived from the mass spectra, suggesting that identification of unknown compounds may be possible.

In a detailed study concerning *N*-methylcarbamates, the performance of API techniques was evaluated through a comparison with the more established TSP and PB interfaces [65]. Mass spectra of methomyl obtained with the various interfaces in the PI mode indicate the advantage of obtaining structural information with API and PB as compared to TSP techniques. In Fig. 15.13, the LC separation of eight *N*-methylcarbamate pesticides with detection by UV and by ion-spray, APCI, PB and TSP MS is shown, illustrating the good responses obtained with both API techniques. Results obtained with the PB interface are not encouraging, probably due to low transport efficiency of the analytes through the interface. In Table 15.2, the detection limits of carbofuran, carbaryl and aldicarb obtained with the various interfaces in this study are compared with literature data. It is clear that APCI using the heated pneumatic nebulizer interface represents a significant advance in terms of sensitivity over the earlier LC-MS approaches. The suitability of LC-APCI-MS for environmental analysis is illustrated by the analysis of a green pepper extract spiked at the 0.1 ppm level with methomyl, aldicarb and carbaryl (Fig. 15.14). The good analyte detectability and the high information content of the mass spectra lead to the expectation that the API technique will become a powerful tool in environmental analysis.

15.4. CONCLUSIONS

In general, on the basis of the results obtained with the various interfaces in LC-MS analysis of pesticides, the conclusion can be drawn that all the interfaces discussed perform satisfactorily, depending on the demands placed on the analysis. Limits of detection that can be obtained are influenced by factors such as the application of sample pretreatment techniques and acquisition of data under SIM conditions. Good examples of the benefits of employing a sample preconcentration step in the analytical method are described by Bellar and Budde [48] for the TSP interface and by Clark et al. [23] for the PB interface; concentration detection limits are in the low-ppb range. In both cases off-line sample preconcentration procedures have been used, which may lead to contamination, analyte losses due to sorption to vessel walls and evaporation and loss of sensitivity because mostly not all of the concentrated sample extract is injected into the analytical system. In addition, off-line preconcentration procedures are time-consuming and difficult to automate. It is clear that these disadvantages can be avoided by incorporation of an on-line sample preconcentration step into the total analytical set-up, which is described by Bagheri et al. [52]. Acquisition of data under SIM conditions in combination with on-line sample preconcentration resulted in concentration detection limits in the ppt range.

TABLE 15.2

DETECTION LIMITS (ng) AND QUANTIFICATION IONS FOR THREE REPRESENTATIVE CARBAMATE PESTICIDES USING VARIOUS LC-MS SYSTEMS [65]

Carbamate	MW	Moving belt (EI) SIM	DLI Full-scan (150–550)	Detection limit (ng) (quantitation ion)						
				TSP		PB-EI		Ion- spray SIM	APCI	
				Full-scan (60–550)	SIM	Full-scan (62–450)	SIM		Full-scan (50–350)	SIM
Carbofuran	221	25 (165)	50 (222)	2 (239)	0.8	240 (164)	55	0.3 (222)	0.5 (222)	0.05
Carbaryl	201	25 (145)	40 (202)	4 (219)	0.8	100 (144)	10	1.0 (202)	0.5 (145)	0.05
Aldicarb	190	6 (89)	60 (191)	4 (208)	0.9	NA (89)	>500	1.5 (191)	1.0 (116)	0.07

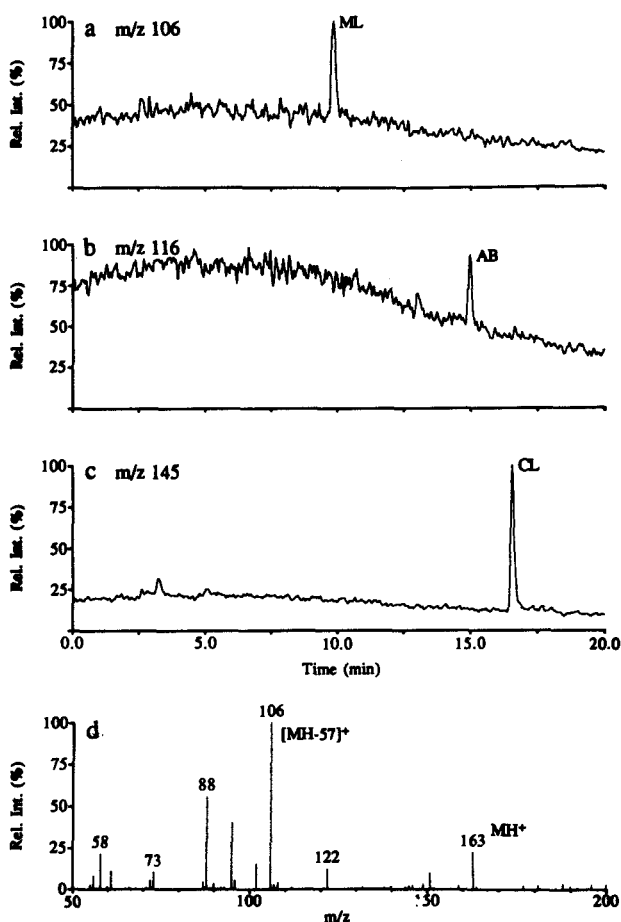


Fig. 15.14. Analysis of spiked (0.1 ppm) green pepper extract by LC-APCI-MS. Mass chromatograms of (b) the $[MH - 75]^+$ ion of aldicarb (m/z 116) and $[MH - 57]^+$ ions of (a) methomyl and (c) carbaryl (m/z 106 and 145, respectively), extracted from full-scan (50–350 amu) TIC; (d) background-subtracted spectrum of methomyl taken from top of peak in (a) [65].

In environmental analysis, however, sensitivity is not the only requirement for analytical method development. Besides confirmation of the identity of target compounds, identification of non-target analytes is an important aspect. When LC-MS is to be used as an identification technique, the possibility of acquiring structural information is an inevitable requirement which is not met by every interface. With the helium jet DLI and the TSP interface, the information that can be derived from the mass spectra is limited to the molecular weight of the compound under investigation, due to the soft nature of the ionization process, although in some cases occasional fragment ions resulting from the loss of a functional group may be observed. Despite various efforts to obtain more

structural information, such as the addition of chloroacetonitrile to the mobile phase for CLENI, employing both positive and negative ion modes, carrying out postcolumn extraction procedures or the use of various buffers in the case of TSP-MS, no significantly different information concerning the structure of the compounds becomes available. In addition to this, the application of the helium jet DLI interface is confined to micro-LC, which is in most cases not the method of choice for environmental analysis, due to the restricted sample capacity. Although satisfactory results have been obtained with the helium jet DLI interface in some cases, DLI is not used anymore, which can be concluded from the lack of recent publications.

Of all the interfaces described for environmental analysis by means of LC-MS, the TSP interface is the most widely used, and the results obtained with it are very good in terms of sensitivity and confirmatory power. In developing a broad-spectrum analytical method for the determination of non-volatile compounds in aqueous environmental samples [48], it was concluded that the combination of precise retention times, molecular weight information, evidence from ions containing naturally occurring isotopes, and occasional fragment ions, gives reasonable information for the identification of target analytes. However, the thermospray technique has proven not fit for identification purposes.

The PB interface, which can provide structural information derived from EI mass spectra, enables identification of non-target analytes. In most cases, detection limits obtained are not as low as those obtained with the TSP interface, but low enough to make the PB interface suitable for environmental analysis. A major point of concern in the application of the PB interface is the analyte transfer process in the interface, which is assumed to be the most important factor associated with the non-linearity of the analyte signal and responsible for the large variation in detection limits reported in the literature.

API techniques, and especially APCI, introduced only recently for the determination of pesticides, seem to be a very good alternative both in terms of detection levels, i.e. in the low-nanogram range, and structural information, with the possibility of identification of unknown compounds. Although the results reported so far are very promising, it is still too early to draw conclusions.

The conclusion that LC-MS is a powerful tool in environmental analysis, with the possibility of obtaining adequate detection limits for trace level analysis, seems justified. When PB or API interfacing techniques are used, identification of non-target analytes, often required in environmental analysis, is among the possibilities of LC-MS. In the future, investigations concerning the incorporation of on-line clean-up and preconcentration techniques – in order to further improve analyte detectability, reduce analysis times and allow automation – should be emphasized. With this approach, the benefits of mass spectrometric detection in combination with liquid chromatography can be utilized optimally for routine trace level determination and identification purposes.

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APPENDIX: LIST OF ABBREVIATIONS

APCI, atmospheric pressure chemical ionization; API, atmospheric pressure ionization; CI, chemical ionization; CID, collision induced dissociation; CLENI, chloride enhanced negative chemical ionization; DLI, direct liquid introduction; EI, electron impact; GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; NCI, negative chemical ionization; NI, negative ion; PB, particle beam; PCI, positive chemical ionization; PI, positive ion; SIM, selected ion monitoring; TIC, total ion current; TSP, thermospray; UV, ultraviolet.

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Chapter 16

Hyphenated techniques applied to the speciation of organometallic compounds in the environment

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16.1. INTRODUCTION

The fate of trace metals and their impact on the environment have been a major concern for more than 20 years. Most studies have been limited to total metal determination. In general, organometallic compounds represent a small fraction of the total metal burden in the sample. However, organometallic forms of metals are usually much more toxic than their inorganic counterparts.

Organometallic compounds occur in the environment as a result of direct anthropogenic inputs or because they are naturally formed there. They are used in a wide variety of industrial processes, sometimes at the percent level. Some organometallic forms of mercury, arsenic and now tin have been observed to have efficient biocidal properties. This fact has largely been applied to the synthesis of a large variety of pesticides, leading then to direct introduction in the environment. Another well-known source is directly related to the widespread use of methyllead ions as antiknocking additives in gasoline, resulting in an important global dispersion of lead species.

Natural methylation in the environment is now well established for a number of elements. Mercury was one of the first cases studied owing to its methylation potential and the high toxicity associated with the final product. Natural alkylation of arsenic and tin are now well-established facts. Analytical or chemical evidence of environment methyl-metal formation also exists for selenium, tellurium, and germanium. Antimony methylation is now questioned due to possible analytical errors. Lead methylation is still controversial.

Except when under the influence of direct anthropogenic inputs, organometallic compounds occur in the environment at very low, ultra-trace levels. The yield of natural methylation mechanisms is always small. As a result, methylated species represent only a few percents of the total metal concentration in water and sediments. However, through bioaccumulation processes in the food chain, methylated forms of metal (e.g. Hg) can represent up to 90% of the total metal concentration present in fish flesh.

One of the first obvious concerns in addressing organometallic determination in the environment is to be able to detect and quantify species. The major analytical challenges are then sensitivity and selectivity. Both requirements are far from satisfactorily met by most present commercial instrumentation. This problem has been solved most generally by using a combination of different analytical processes and analytical techniques. Most successful combinations result from the interface between chromatographic techniques (gas or liquid providing the "species differentiation ability") to atomic spectrometry detectors (accounting for selectivity). Their hyphenation allows mastery of the technical skills necessary to perform organometallic determination. It has, however, also multiplied the analytical steps in the procedure. We can say that part of the success of speciation techniques lies in the quality and efficiency of the interface design between the different stages of the analytical procedure. In most cases, analytical speciation schemes rely on the combination of three basic steps and their interfacing design at the instrumental level:

- analyte preconcentration
- separation (chromatographic or differential concentration mode)
- selective detection (single or multi-elemental)

All techniques first require a preconcentration step. This procedure can be performed off-line from the instrumentation, or on-line as a part of the instrumentation set-up.

The first range of techniques using off-line preconcentration most often use standard chromatographic procedures (gas or liquid). The sample injection mode requires only a small volume of sample (a few microlitres). For both the gas or liquid separation approach, a very important preconcentration step is required prior to injection. Interfacing is in general straightforward and the gaseous nature of the effluent can be easily adapted to various atomic spectrometry techniques such as atomic absorption, atomic emission, atomic fluorescence or even mass spectrometry. Liquid chromatography separation techniques are very powerful as they generally allow the derivatization stage to be by-passed and provide a large panel of possible chromatographic procedures enabling a larger range of organometallic compounds to be determined. However, the small amount of eluent delivered leads to an overall low sensitivity. Interfacing is more complex in this case since the liquid eluent must be transformed into the gaseous state prior to detection. This stage is technically difficult and usually leads to an overall loss in sensitivity (Fig. 16.1).

An on-line technique using direct interfacing between hydride generation methods, and combining simultaneously the preconcentration step by cryofocusing and later chromatography by gentle warming of the trap can be very easily interfaced with atomic absorption spectrometry or atomic fluorescence. Sample volumes are larger in this case and range from 10 to 1000 ml. Techniques derived from these procedures achieve the highest sensitivity and ease of operation (Fig. 16.1) in comparison to other methods using off-line preconcentration methods with considerably fewer analytical steps. However, they are limited to low boiling point organometallic species (methyl, ethyl, butyl) and have a low resolution capacity. Nonetheless, the high sensitivity of these techniques has indicated the

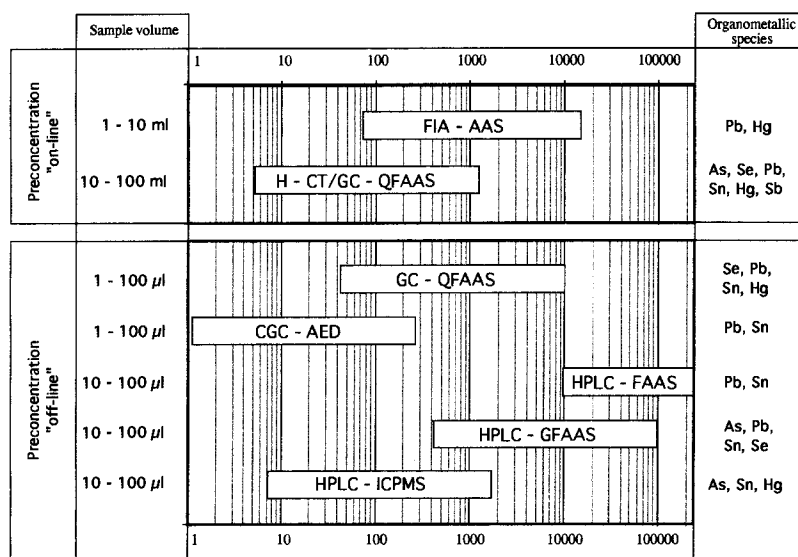


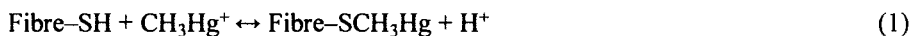
Fig. 16.1. Detection limit ranges of speciation techniques coupled to atomic spectroscopy detectors (in pg).

occurrence of unexpected species in the environment. In general, absolute detection limits of these hyphenated systems are always at the picogram level since they classically represent the detection limits achieved with the atomic spectrometry detectors. In all cases, the quality of the preconcentration step is of vital importance since it then provides the suitable sensitivity for working conditions associated with environmental analysis.

16.2. FLOW INJECTION ANALYSIS

Flow injection analysis (FIA) has not been widely applied to the determination of organometallic compounds. Flow injection methods have more often been applied to the differentiation of redox species such as $\text{Cr}^{\text{III}}/\text{Cr}^{\text{VI}}$, $\text{Fe}^{\text{II}}/\text{Fe}^{\text{III}}$, $\text{As}^{\text{III}}/\text{As}^{\text{V}}$ and $\text{Se}^{\text{IV}}/\text{Se}^{\text{VI}}$. Speciation using this technique should primarily be understood as a method for differential determination using on-line discriminating chemical reactions rather than as a method based on true chromatographic separation properties. However, there is much value in considering FIA introduction techniques hyphenated to atomic spectrometry. Because of the potential for performing on-line preconcentration and separation reactions, and the fact that these techniques can be very simply interfaced with a wide range of atomic detectors such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma/atomic emission spectrometry (ICP/AES) or inductively coupled plasma/mass spectrometry ICP/MS [1]. Straight interfacing between the FIA device and the spectrometer, through standard nebulization devices, can easily be achieved. Also, the use of a gas liquid separator after on-line hydride generation reactions (or reduction to the metallic state, as for Hg) allows direct introduction of the sample, in a gaseous state, into the atomic detector. Detectors adapted to flow injection techniques in trace-metal speciation easily match the continuous flow characteristics of the technique. Flame atomic absorption spectrometry (FAAS) and inductively coupled plasma coupled to atomic emission spectrometry (ICP/AES) have been extensively used. The sensitivity required for speciation analysis is obtained by direct selective preconcentration on micro-columns. The low contamination and the possibility of on-line matrix pretreatment lead to an excellent overall reproducibility (0.5–1%). Analytes can be concentrated from millilitres of sample down to few microlitres to be injected into the detector, allowing concentration factors of 50 to 100.

Speciation conditions can be achieved using several continuous separation techniques such as dialysis, gas diffusion, ion exchange, liquid-liquid extraction or redox reactions. Despite the versatility and potential of FIA introduction methods, very few reports exist on the speciation of organometallic compounds. Here we present two very different approaches, illustrating the potential of FIA techniques when applied to the determination of organometallic compounds. Speciation between inorganic and methylmercury can be obtained using the differential affinity of sulphhydryl cotton to methylmercury under various pH conditions according to the following reaction [2]:



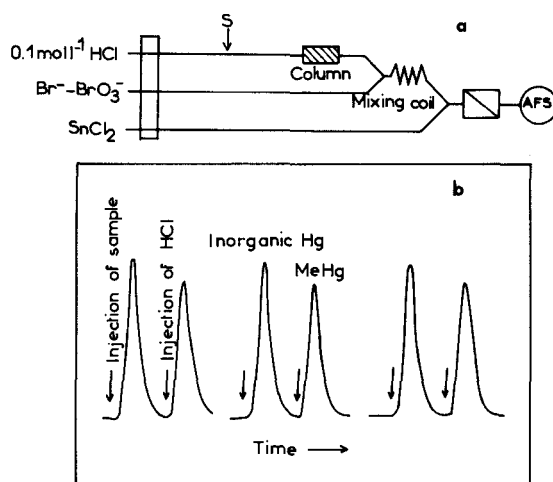


Fig. 16.2. Speciation of Hg by flow injection and detection by atomic fluorescence (FI-AFS). (a) Manifold design, MeHg^+ in comparison with inorganic Hg is retained on the sulphhydryl cotton microcolumn and undergoes desorption and additional postchemical reactions before AFS detection (after ref. 2). (b) Reproducibility of FI-AFS, $\text{Hg}^{\text{II}}/\text{MeHg}^+$ solution 2 ng l^{-1} (courtesy of C.W. McLeod & W. Jian, Sheffield Polytechnic, UK, presented at the 1990 Winter Conference on Plasma Spectrochemistry, St. Petersburg, USA, January 1990).

A mixed solution of inorganic and methyl-mercury is flushed onto a microcolumn packed with sulphhydryl cotton in the manifold presented in Fig. 16.2. Inorganic mercury is not retained and is reduced to elemental Hg^0 by a SnCl_2 solution.

After recording the inorganic mercury peak, the elution of methylmercury occurs upon acidification of the microcolumn. On-line bromination and further reaction of methylmercury with SnCl_2 generates Hg^0 which will be detected in the atomic fluorescence apparatus. The sulphhydryl microcolumns have been adapted for selective field-preconcentration of methylmercury in natural river waters at the sub- $\mu\text{g l}^{-1}$ level [2].

The speciation of tetraalkyleads (tetramethylead (TML) and tetraethyllead (TEL)) using an FIA manifold has also been reported [3]. In this case, the speciation approach is different. It does not rely on the selective retention properties of an adsorbent material as described above but is based on the differential atomization efficiencies of the TML and TEL in the flame of an AAS. The FIA manifold is interfaced to an atomic absorption spectrometer with a fuel-lean acetylene flame. Since TML gives higher absorbance peaks than TEL for a given sample, the experimental absorbance peak recorded can then be related to the respective concentrations of TML and TEL according to the following equations [3]:

$$h_t = (h_1 + h_2) = (a_1 + a_2)/2 + (b_1 \times C_{\text{TEL}}) + (b_2 + C_{\text{TML}}) \quad (2)$$

$$C_T = C_{\text{TEL}} + C_{\text{TML}} \quad (3)$$

where h_i is the measured experimental peak height, h_1 and h_2 are the values corresponding to each compound (not obtained separately), a_1 and a_2 refer to the intercepts of the calibration curve from TML and TEL, respectively, and b_1 and b_2 to the corresponding slope values. The total lead value, C_T is obtained separately on the same manifold, using an on-line demetallation of the tetraalkylleads by a solution of iodine in petroleum spirit (6% w/v) [3]. Limits of detection obtained for total Pb determinations are $43 \mu\text{g ml}^{-1}$ and $0.6\text{--}0.8 \mu\text{g ml}^{-1}$ for TML and TEL, respectively. The future of FIA for the speciation of organometallic compounds can be predicted. If manifolds and detectors can easily be assembled for this purpose, the most promising steps will certainly arise with FIA being used as an "on-line" sample preparation procedure prior to the necessary steps to perform the speciation of the organometallic moieties. As an example, there is the possibility of coupling flow injection (FI) to high performance liquid chromatography (HPLC) for on-line sample clean-up, precolumn derivatization, and the elimination of matrix effects. Possibilities of interfacing FI to hydride generation/cryogenic trapping/gas chromatography/quartz furnace atomic absorption spectrometry (H/CT/GC/QFAAS) also exist, and need to be explored. In general, the automation of FI-hyphenated systems will allow higher sample through-puts. Further, if species could be preconcentrated from the sample, in the field, under good conditions, with stable storage on the same solid support, and later eluted directly into a FI-hyphenated system, this could change the prospects of environmental speciation analysis, reduce sample sizes and the analytical errors associated with sample contamination through handling.

16.3. VOLATILIZATION REACTIONS, CRYOGENIC TRAPPING, CHROMATOGRAPHIC SEPARATION AND ATOMIC SPECTROMETRY DETECTION

Similar to flow injection methods, the technique combining on-line hydride generation, cryofocusing and chromatographic separation prior to detection by atomic spectrometry has received considerable interest during the last 10 years. This hyphenated technique was first used in 1975 for the determination of methylated forms of selenium in freshwater environments [4]. Since then, it has been used for the speciation of arsenic, antimony, selenium, germanium and recently mercury. In recent years, it has been extensively applied to the determination of organotin, and more specifically butyltin compounds, in the environment because of its simplicity and high sensitivity. The on-line integration of the different steps gives the technique a wide range of applications. All types of samples have been approached with this technique: air, water, sediments and biological tissues.

In comparison with standard hydride generation methods, the introduction of the cryogenic and chromatographic separation steps provides both the high sensitivity and speciation ability required for environmental applications. In addition to the direct gain in sensitivity achieved by hydritization and cold trapping for elements such as As, Bi, Sb, Se, Sn, Ge and Te, the system provides the potential for redox speciation of inorganic species of As (III and V), Sb (III and V) and Se (IV and VI). It is also very efficient for most low boiling point alkylated species of environmental significance [5]. Organogermanium compounds, such as the mono-, di- and trimethylated forms, have been reported to occur in

oceans [6]. Arsenic and selenium species frequently detected by this technique include monomethylarsonate (MMA), dimethylarsinate (DMA) species and dimethylselenide, dimethyldiselenide and diethylselenide. Both these elements have higher organometallic forms that are not directly amenable to gaseous derivatization methods. Species such as arsenobetaine, arsenocholine, arsenosugars for arseno- or selenomethionine or selenocysteine, require a wet digestion and determination as the inorganic form. HPLC combination methods are better suited for the direct species separation and are described later in this chapter. Applications of cryogenic systems have gained increasing popularity for the determination of organotin compounds. Methyl-, ethyl-, and especially butyltin (including tetrabutyltin) species have received considerable attention in the last few years in a wide range of samples [7]. Finally, mercury (inorganic and methyl-, dimethyl-, diethylmercury) and lead (inorganic and methyl- and ethyl- lead) species can be detected with a slightly modified version of the instrumentation [8,9].

16.3.1. System design

This technique combines four basic stages: on-line aqueous derivatization of the analytes; preconcentration by cryofocusing; chromatographic separation; detection by atomic spectrometry. The most frequently used detectors are atomic absorption (AAS), atomic fluorescence (AFS) in the particular case of mercury, or sometimes mass spectrometry (MS). The success of the technique lies in the compactness of the system design, the integration on-line of the different analytical steps, and the potential for addition of prior sample pretreatment procedures. Details of the analytical system are presented in Fig. 16.3. Hyphenation between the different analytical stages is very simple, since after the volatilization the analytes are processed and introduced into the detector in the gaseous state. However, a careful design of the system is necessary to minimize the dead volume and to give optimum working conditions. The reaction vessel for the derivatization reaction is usually constructed in borosilicate glass. The volume is highly variable and can range from a few millilitres to 500 ml, according to the type of species and sample investigated. The reaction flask should be shaped so that entrapment of volatile species can be avoided [10,11]. The bubbler can either enter the sample or lie slightly above it (foaming samples) [7]. Passivation of the glass surfaces was only found to be necessary for organogermanium determinations [6,12]. Recent improvements in the hydride generator design have been proposed. The reactor is composed of a Büchner funnel and an outer glass cylinder, and the reaction takes place in the funnel where the sample and reagents are mixed. The carrier gas flows continuously through the fritted disc and sweeps the hydrides to the detectors. This simple, continuous hydride generator combines hydride production and gas-liquid separation steps, leading to an improved signal to noise ratio and efficiency in stripping the hydrides from the solution [13]. Removal of the water generated during the derivatization reactions may be necessary and improve the reproducibility in performance of the instrumentation. Solid drying agents are to be avoided since they will also irreversibly trap some of the analytes. Cryofocusing of the analytes is performed in a small (30–45 cm long, 6 mm i.d.) packed chromatographic column immersed in liquid nitrogen (−196°C). This cryofocusing trap is also used as a limited chromatographic separation column after it is removed and heated by a Nichrome wire. Despite its



Fig. 16.3. Hyphenated system for the speciation of As, Se, Hg, Se and Pb developed at the University of Bordeaux I. (a) Derivatizing reagent storage flask (NaBH_4 or NaBEt_4); (b) reaction flask (a and b are adapted from a PE MH 20 hydride generation system); (c) three-way valves; (d) trapping column packed with chromatographic material and heating wire; (e) removable liquid N_2 Dewar; (f) quartz furnace atomizer; (g) electrothermal furnace; (h) atomic absorption apparatus (PE 5000).

low resolution ability (1300 theoretical plates [7]), the quality of the packing phase is of prime importance for the reproducibility and peak resolution. However, such a simple chromatographic step is quite sufficient to separate most organometallic compounds of interest (one to eight species can be present on a single chromatogram). The quality of the chromatography support, mesh size, and quality of the stationary phase are critical. Mesh sizes of 80–120, with 10–20% loading of non-polar stationary phases (silicone based, such as OV 1, OV 3, OV 101, SE 50, SE 54, and SP 2100) have been used for the determination of methylated tin, alkylleads and methylated mercury species [8,14–19]. Lighter loadings (3–5%) are necessary for methylated arsenic [20] or selenium species [21] and higher boiling points species such as butyltin compounds [22]. In some systems, a preconcentration stage prior to the chromatographic separation has been reported. Trapping of volatile organotin compounds at room temperature can be performed on Tenax GC for preconcentration purposes [23,24]. Derivatized ethylated mercury species can be trapped and separated on Carbotrap columns [25].

The on-line preconcentration and separation of derivatized species is one of the major advantages of the H/CT/GC/QFAAS method. Cryofocusing allows preconcentration factors of 50–100-fold. Separation is achieved upon warming of the trap and species are eluted on the basis of their boiling points and according to their chromatographic properties. The combination of this technique with the highly specific detection by atomic absorption spectrometry using deuterium background correction allows selective recording of inorganic and organometallic forms of the metals present in the samples on the same chromatogram (Fig. 16.4). In general, the reproducibility with such systems ranges be-

tween 5 and 15% ($n = 5$). Careful automation of each analytical stage should considerably reduce the relative standard deviations obtained routinely. A minimal dead volume is essential for high sensitivity and low tailing of the chromatographic peaks. When the system is fully optimized, absolute detection limits of some 10–100 pg of metal can be obtained for each organometallic species analyzed [14]. The combination of these low detection limits with the possibility of using large reaction vessels (100–500 ml) allows the direct determination of traces of organometallic compounds in environmental waters at the ng l^{-1} level.

16.3.2. Derivatization reactions

The first step of the technique is the extraction of the analytes from the sample matrix by volatilization reactions under acidic conditions. This step is of prime importance as it serves many purposes. In addition to removing the analytes from the matrix, it also allows the on-line preconcentration which is fundamental for obtaining the appropriate sensitivity for environmental analysis. Also, if the derivatization reaction is performed under good conditions, it will minimize the elements trapped on the column and thus simplify the potential matrix interferences at the later detection stage. Hydride generation has long been the most popular method for converting the analytes into the gaseous state. Hydride generation methods have been extensively used for the determination of the inorganic species of As^{III} and As^V , Sb^{III} and Sb^V [20] and Se^{IV} and Se^{VI} [21] by selecting appropriate pH ranges for reaction. Inorganic tin species (Sn^{II} and Sn^{IV}) cannot yet be differenti-

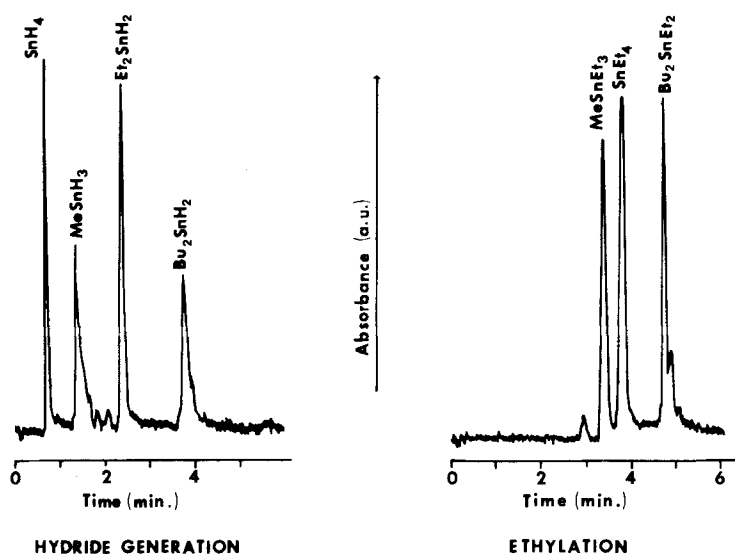
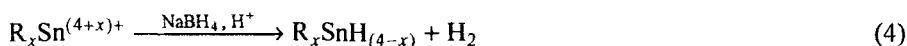


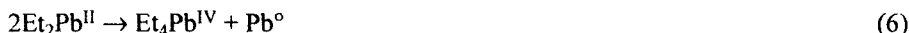
Fig. 16.4. Chromatograms of organotins compounds (5 ng as Sn in a 100-ml reaction flask) after derivatization by $NaBH_4$ or $NaBEt_4$, cryogenic trapping, gas chromatography separation and detection by atomic absorption (F.M. Martin and O.F.X. Donard, unpublished results).

ated by these approaches. Hydride generation has also proved to be very efficient for the quantitative conversion of low organometallic forms of Ge, Se, Sb, As and Sn [5]. The pH conditions for reaction are critical for a quantitative conversion of the analytes (Table 16.1). In general, comparison of the pH and the pK_a of the species shows that the reduction is generally performed at a pH a few units below the pK_a of the species of interest [26]. The general reaction can be described for alkylated tin compounds [15]:



with $x = 1, 2, 3$ and R is methyl, ethyl or butyl.

Hydride generation was not considered to be suitable for environmentally important methylated forms of Hg and Pb. The field of application of the D/CT/GC/AAS (derivatization/...) method has now been extended to Hg and Pb, as well as to their alkylated derivatives, by the use of a new boron alkylating reagent $NaBEt_4$ [8,27]. Thus Hg and Pb can be quantitatively ethylated at pH 5 in water according to the following reactions:



The application of this new derivatization method is under current development. It has been shown to be less sensitive to interferences than hydride generation for the determination of butyltin compounds in complex sediment matrices after an ethanolic extraction step [28]. The role of the derivatizing reaction is of prime importance. This is true for all techniques reviewed in this chapter, but here it facilitates the integration of many analytical steps required for the speciation of organometallic compounds. Further, derivatizing reactions which produce gaseous analytes from the aqueous solution also selectively limit the number of other species which could possibly interfere at the later detection stage. Finding new derivatizing reagents to achieve volatilization of the analytes will most probably be important in improving techniques which use cryogenic trapping for separations. Recently, $NaBH_4$ and $LiB(C_2H_5)_3H$ have been reported to convert mercury(II) and methylmercury(II) quantitatively into volatile forms as the hydride [29,30].

16.3.3. Atomization reactions and detection

The continuous gaseous effluent coming from the chromatographic column needs to be readily atomized in the beam of an atomic spectrophotometer. A flame, graphite furnace, or electrothermally heated cell are most frequently used for detection. The "central problems in spectrochemical analysis are associated with atomization processes" according to Sir A. Walsh [31]. This part of the detection mode, usually performed with an atomic absorption spectrometer, is indeed critical to yield optimum sensitivity. With most hyphen-

TABLE 16.1

DERIVATIZING CONDITIONS FOR THE SYSTEMS USING
CRYOGENIC/CHROMATOGRAPHY SEPARATION PRINCIPLES

Species	Reagent	Derivatization conditions	Sample pretreatment	Ref.
TRISn ^a Me _x Sn ^{(4-x)+} Et _x Sn ^{(4-x)+} n-Bu _x Sn ^{(4-x)+}	NaBH ₄	1 ml of 4% aqueous NaBH ₄	1 ml of 2 M acetic acid	16
TRISn Me _x Sn ^{(4-x)+}	NaBH ₄	2 × 1 ml of 1% aqueous NaBH ₄	pH 6.5 with 4 ml of 2 M Tris-HCl	17
TRISn Me _x Sn ^{(4-x)+}	NaBH ₄	1 ml of 4% NaBH ₄ in 0.02 M NaOH.	pH 2 with 0.2 ml of 5 M HNO ₃	18
TRISn Me _x Sn ^{(4-x)+} n-Bu _x Sn ^{(4-x)+}	NaBH ₄	2 × 1.5 ml of 4% aqueous NaBH ₄	pH 2 with 0.2 ml of 5 M HNO ₃	15
n-Bu _x Sn ^{(4-x)+} Et ₃ Sn ⁺	NaBH ₄	2 × 2.5 ml of 6% aqueous NaBH ₄	pH 1.6 with 2ml of 5 M HNO ₃	22
Ge Me _x Ge ^{(4-x)+}	NaBH ₄	6 ml of 20% NaBH ₄ in 0.06 M NaOH per 100 ml of sample	5 ml of 1.9 M Tris- HCl + 10 ml of 300 g/l NaCl + 1 ml of 0.2 M EDTA per 100 ml of sample	6
As ^{III}	NaBH ₄	2 ml of 2% aqueous NaBH ₄	1-3 ml of 5% potassium biphtalate pH 3.5-4	19
As ^V MMA DMA Trimethyl- arsine	NaBH ₄	4 × 2 ml of 2% aqueous NaBH ₄	pH 1-1.5 with 5 ml of saturated solution (10% w/v) of oxalic acid in water	
Me _x Pb ^{(4-x)+}	NaBEt ₄	3 ml of 0.43% NaBEt ₄ in water	pH 4.1	8
MeHg ⁺	NaBEt ₄	50 μl of 1% NaBEt ₄ in water	pH 4.9 with an acetate buffer solution	25
Hg ²⁺	NaBH ₄	1 ml of 0.4% aqueous NaBH ₄	pH 4	29
MeHg ⁺	LiB(C ₂ H ₅) ₃ H	0.1% solution of LiB(C ₂ H ₅) ₃ H in THF	pH 4	29

^aTRISn, total recoverable inorganic tin.

ated systems, the molecular identity of the analytes must be preserved for their separation by chromatographic procedures. However, since most techniques are coupled to atomic spectrometry, to obtain good selectivity and sensitivity, the alkylated species injected into the detectors must be efficiently dissociated to produce a high density of atoms in the de-

tection cell of the spectrometer. The details and discussion presented in this section are directly relevant to speciation systems using derivatization, cryogenic trapping and separation as a separation unit, but are also directly applicable to systems based on gas chromatography or liquid chromatography followed by on-line volatilization, as described later in this chapter.

At first, the interfacing between a gas chromatograph and an atomic absorption spectrometer used direct injection of the gaseous analytes into the nebulizer of a flame AAS. Some of the first reports of such interfacing and atomization methods were those by Kolb et al. [32] who directed the effluent of a gas chromatograph to the nebulizer of an atomic absorption spectrometer for the determination of alkylleads in gasoline. Similar atomization procedures have been used for the determination of organosilicon compounds [33]. However, these resulted in an overall poor sensitivity, which was possibly related to poor atomization mechanisms and a short residence time of the generated atoms in the light-path of the spectrometer. Further, the combined use of the nebulizer and burner led to considerable broadening of the individual chromatographic peaks. Improvements in sensitivity were obtained by using ceramic tubes suspended above the conventional flame burner, resulting in an increase in the density of the atomic cloud and in its residence time along the optical axis of the spectrometer [34].

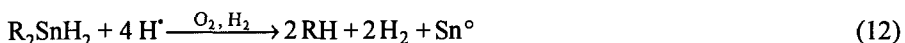
The graphite furnace has also been seldom used as a means of atomization after GC separation [35–37]. A simple heated stainless steel transfer line can connect the outlet of the gaseous separation device and inject its effluent directly into the graphite tube. The graphite tube can be fired sequentially, within the elution interval of the organometallic species for germanium species [6], or continuously during the chromatographic elution. These repeated firings considerably reduce the lifetime of the graphite tube which is expensive. The atomization mechanisms associated with this type of atomizer are believed to involve surface reactions originating on the graphite walls [35]. The decomposition of alkylated species occurs in this case well below the volatilization temperature of the metal. The addition of H_2 to the argon purge gas stream resulted in improved detection limits, probably by providing a source of active radical scavengers improving the overall atomization efficiency.

An efficient and inexpensive alternative to the graphite furnace atomizer uses a silica furnace, heated electrothermally. Some early uses of this atomization device were for arsine [38] and selenium species after packed column chromatography [39]. A large variety of designs has been used including one with a cell path-length of 1 m [40]. A typical configuration is presented in Fig. 16.5. Some of the important features of this atomizer are associated with the additional introduction of H_2 and O_2 gas to enhance the atomization efficiency, and the use of a narrow beam width. Both gases are reported to yield efficient atomization processes although the detailed mechanisms are not yet confirmed. Dedina and Rubeska [41] were among the first to suggest the idea of atomization of SeH_2 via free radical mechanisms and H_2 was indeed found to be essential to generate atomization. Most of the time, these quartz furnaces are operated at temperatures ranging from 700 to 950°C, which is far below that required for atomization of selenium or arsenic hydrides in graphite tubes [42,43]. It is generally accepted that atomization in the silica furnace proceeds in the presence of H_2 and O_2 via chain reactions which lead to the formation of H^\bullet or OH^\bullet radicals according to the following reactions [14]:



An important feature of the design of the quartz atomizer results from the necessity of delivering the mixture of H_2 and O_2 with the analytes in the carrier gas exactly at the intersection with the beam of the spectrometer. A small flame is burning and H radicals are formed either in the flame or at the beginning of the hot zone of externally heated atomizers [44].

For organometallic species, the decomposition of the molecule proceeds via a succession of electrophilic/nucleophilic reactions of the polarized hydrogenated alkylated molecule with the OH or H radicals. Atomization can be described as follows for a dialkyltinhydride [14]:



It is important to have some knowledge of these mechanisms since interferences at the atomization stage can sometimes occur during the analysis of complex environmental matrices. These interferences can be generated when compounds co-eluting with the analytes from chromatography are present in excess over the analytes or are decomposed faster by H^{\bullet} radicals attack. Both processes lead to an overall depletion of radicals available for the atomization of the organometallic species and result in signal depression. In general, however, the use of a background correction lamp is not necessary, resulting only in the alteration of the signal-to-noise ratio.

The lifetime of the quartz tube atomizer is variable and can be several months. New tubes initially perform poorly and must be conditioned by several runs before giving optimum sensitivity. Contamination of the quartz surface may induce severe signal suppression.

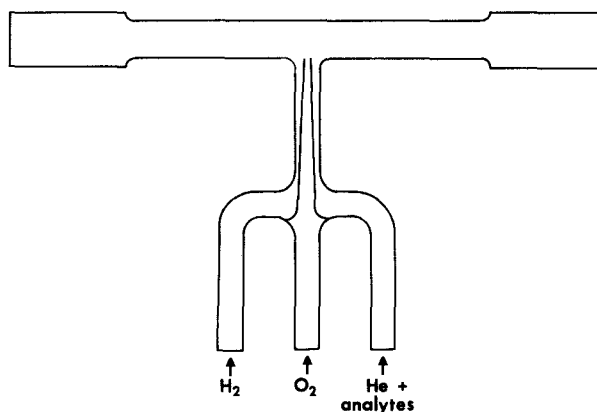


Fig. 16.5. Quartz atomizer design (light path length = 19.5 cm; internal diameter = 0.8 cm).

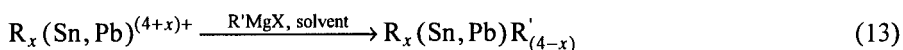
sion in some cases. Conditioning of the furnace by treatment with 40% HF for 15 min will improve its efficiency [45]. Devitrification of the quartz inevitably occurs and will affect both the sensitivity and precision. However, the low cost allows simple replacement. The method has now been adapted to a wide range of chromatography systems including those using capillary columns [46]. Despite the few problems mentioned above, the overall efficiency, sensitivity and low cost of the quartz electrothermal atomizer are responsible for its increasing popularity in the field of speciation of several organometallic species of Sn, Se, As, Sb, Pb and Hg.

Other detectors, such as flame photometric detectors (FPD), also provide excellent sensitivity with various chromatographic systems, but are not selective enough [18]. Finally, AFS is the preferred detector for mercury analysis. It is then more sensitive than atomic absorption spectrometry and allows picogram detection limits with the speciation of inorganic and methylated forms of mercury in the environment [25]. Applications of these techniques to the determination of As, Se, Sn and Hg in environmental samples are listed in Table 16.2.

16.4. SEPARATION BY GAS CHROMATOGRAPHY

Gas chromatography has been interfaced successfully with a wide range of detectors, since the analytes arrive at the detector in the gaseous state. Interfacing is straightforward; in most cases a simple heated stainless steel transfer line will bring the gaseous analytes to the detectors. However, despite its apparent simplicity, this connection is critical, and needs to be mastered to achieve good separation and detection [51]. It is most important to have well-designed and well-manufactured transfer lines. They should be as short as possible and heated to avoid condensation and decomposition of the analyte. Most of the original systems used packed-column chromatography but they are now usually replaced by high resolution capillary gas chromatography (HRCGC). HRCGC has probably indirectly increased the sensitivity of the method. Increased separation of compounds which are likely to co-elute with the organometallic species avoids competition later in the atomization and detection processes. However, the sharp peaks obtained make heavy demands on the detector's speed of data acquisition.

The absolute detection limits obtained with these systems are similar to those using on-line preconcentration since they very often use the same detector, that is atomic absorption. However, the important difference lies in the initial sample volume. With on-line hyphenated systems, the sample volumes can be as large as several hundreds of millilitres if required. Off-line hyphenated systems can only inject a few microlitres into the gas chromatograph and therefore require an important preconcentration step of the analytes prior to injection. Extraction from the sample and preconcentration then need to be performed off-line using a solvent medium, with or without chelating agents. Difficulties exist in the quantitative liquid/liquid extraction of all inorganic ionic and organometallic species. Several procedures have been developed to derivatize and concentrate the analytes after extraction from the sample, and prior to chromatographic separation. In most cases, organolead or organotin compounds react with a Grignard reagent to form volatile tetraalkyl derivatives which are suitable for chromatography.



with $x = 1, 2, 3$ and R is inorganic Sn, methyl, ethyl, butyl or phenyl.

For lead, R' can be propyl, butyl, or phenyl. For tin, a larger variety of R' substituents has been used, such as methyl, butyl, pentyl and hexyl. After derivatization, the excess of Grignard reagent is destroyed by the addition of sulphuric acid and the solution dried by an agent such as anhydrous CaCl_2 . Finally, the sample is concentrated under a gentle stream of nitrogen.

These techniques have been extensively used for environmental contamination problems, with a large variety of samples (air, water, sediments and biological tissues) as shown in Table 16.2. They were principally restricted to the determination of organotin, organolead and organomercury compounds.

The widest array of analytical solutions has certainly been developed for the determination of organotin compounds; their toxicity is well established and the number of compounds under investigation is continuously rising. The protocols for alkyllead determination are very similar to those used for alkyl- or aryltins. Both hyphenated techniques most frequently rely on gas chromatography interfaced to a quartz electrothermal atomizer aligned in the beam of an atomic spectrometer, and use similar derivatization reactions. The analytical solutions used for the speciation of organomercurials are slightly different. After gas chromatography, the organomercurial compounds are usually reduced to Hg^0 and detected in a cold cell by AAS or by AES. This latter detection mode is the most popular for mercury after gas chromatographic separation.

The different approaches used for the speciation of important pollutants such as tin, lead, or mercury compounds are now presented. The emphasis is on the derivatization reactions and sample preparation rather than the interfacing, since hyphenation between gas chromatography and most detectors is either straightforward (as mentioned earlier) or is available commercially such as GC coupled to microwave-induced plasma/atomic emission spectroscopy (GC-MIP/AES).

16.4.1. Determination of organotin compounds

Some of the first methods concerned with the speciation and environmental aspects of tin compounds appeared in the late 1970s and early 1980s. Meinema and his co-workers presented a method for the determination of butyltin compounds by solvent extraction, derivatization with a Grignard reagent, then gas chromatographic (GC) separation and detection by mass spectrometry [52]. Simultaneously, methods for the determination of phenyltin moieties in water, with hydritization by LiAlH_4 , were published, using GC and an electron capture detector (ECD) or a flame ionization detector (FID) [53]. Chau and co-workers [54] then showed that atomic absorption using an electrothermal quartz furnace could be used as an efficient GC detector, which was applied to the determination of methylated tin compounds in environmental waters. Since then, a wide variety of techniques focusing on the application of gas chromatography have been published, concerning a large class of organotin species such as methyl-, butyl-, ethyl-, propyl-, phenyl- and cyclohexyltin compounds in a wide variety of matrices such as natural waters, sediments

TABLE 16.2

APPLICATIONS OF HYPHENATED SYSTEMS USING GAS CHROMATOGRAPHY COUPLED TO ATOMIC SPECTROMETRY

Species	Detectors	Chromatography	Comments	Samples	Ref.
Applications using D/CT/GC/QFAAS					
TRISn Me _x Sn ^{(4-x)+}	QFAAS	Chromosorb GAW-DMCS 45–60, 3% SP2100	DL 20–50 pg as Sn Reagent: NaBH ₄	Water samples Sediment Biota	15
Me _x Pb ^{(4-x)+}	QFAAS	Chromosorb WAW-DMCS 80–100, 10% SP2100	DL 9–10 pg as Pb Reagent: NaBEt ₄	Synthetic solutions	8
n-Bu _x Sn ^{(4-x)+}	QFAAS	Chromosorb GAW-DMCS 45–60, 3% SP2100	DL 11–45 pg as Sn Reagent: NaBH ₄	Water samples Sediment	22
As ^{III} As ^V MMA DMA	QFAAS	Chromosorb WAW-DMCS 30–60, 10% OV-3	DL 200–500 pg as As Reagent: NaBH ₄	Water samples Sediment	46
As ^{III} As ^V MMA DMA	QFAAS	Glass beads (40 mesh)	DL 19–61 pg as As Reagent: NaBH ₄	Water samples Pore waters	47
Hg ^{II} MeHg ^{II} Me ₂ Hg	CVAFS	Chromosorb WAW-DMCS 60–80, 15% OV-3	DL 0.6 pg as Hg Reagent: NaBH ₄	Water samples Biota	25
Me _x Sn ^{(4-x)+} n-Bu _x Sn ^{(4-x)+}	QFAAS	Chromosorb GAW-DMCS 45–60, 3% SP2100	DL 1.1–2.5 ng for 0.1 g of tissue (wet weight) Reagent: NaBEt ₄	Oysters	48

Applications using GC and various detectors

Sn ^{IV} Me _x Sn ^{(4-x)+}	QFAAS	Glass column, 6 mm i.d., 1.8 m long, 3% OV-1, Chromosorb 80-100	DL 100 pg as Sn	Water samples	54
Me ₃ SnCl Et ₃ SnCl Pr ₃ SnCl Bu ₃ SnCl	ECD	Glass column, 3 mm i.d., 1 m long, 20% DEGS-HG, Chromosorb 80-100	DL 1 pg as Sn	Biological materials	87
n-Bu _x Sn ^{(4-x)+}	FPD	CP Sil 5 CB capillary column. 320 μm i.d., 25 m long, film thickness 0.4 μm	DL 0.2 pg as Sn	Marine waters	85
n-Bu _x Sn ^{(4-x)+}	FPD 610 mm filter	Glass capillary column, 0.53 mm i.d., 12 m long, 3.0 μm film of BP-1	DL 1.5 ng as butyltin	Fish Sediment	59
n-Bu _x Sn ^{(4-x)+} n-Ph ₂ Sn ²⁺ n-Ph ₃ Sn ⁺	FPD no filter	Capillary fused silica. 0.2 mm i.d., 25 m long, 0.11 μm film thickness of SE-54	DL 3–25 pg as Sn	Seawater Sediment Biota	98
n-Bu ₃ Sn ⁺ n-Ph ₃ Sn ⁺	ECD	Capillary fused silica. 0.2 mm i.d., 25 m long, 0.11 μm film thickness of SE-54	DL 10–300 pg as Sn	Seawater Sediment Biota	98
n-Bu ₂ Sn ²⁺ n-Bu ₃ Sn ⁺ n-Ph ₃ Sn ⁺	MS	Capillary fused silica. 0.2 mm i.d., 25 m long, 0.11 μm film thickness of SE-54	DL 10-60 pg as Sn	Seawater Sediment Biota	98
MeHgCl EtHgCl PhHgCl	ECD	FSOT column, 13 m × 0.53 mm i.d., 1.2 μm film thickness of Superox- FA	DL 0.7-3.1 pg as Hg	Biological samples	133

TABLE 16.2 (continued)

Species	Detectors	Chromatography	Comments	Samples	Ref.
MeHgCl EtHgCl PhHgCl	ECD	FSOT column, RSL 300	DL 0.3–0.5 pg as Hg	Biological samples	133
MeHgCl EtHgCl	ECD	Packed column AT-1000	DL 4.1 pg as Hg	Biological samples	133
MeHg ⁺ EtHg ⁺	MIP/AES	Packed column, 2 m × 2 mm i.d., 5% DEGS-PS on 100–120 mesh	DL 3–5 pg as Hg halides	Fish tissue	135
MeHg ⁺ EtHg ⁺	MIP/AES	Fused silica capillary column, 12 m × 0.32 mm i.d., 0.25 μm non polar DB-1	DL 0.8–1.3 pg as Hg butylated	Fish tissue	135
MeHg ⁺	ECD and CVASS	Packed column, 1.8 m × 2 mm i.d., 5% DEGS-PS Supelcoport 100 (no)	Not mentioned	Biological tissue	140
MeHg ⁺ EtHg ⁺	ECD	Capillary column, 20 m polar OV-275 (film thickness 0.25 μm)	DL 2 pg as Hg	Fish and mussel samples	134
Me ₄ Pb Me ₃ EtPb Me ₂ Et ₂ Pb MeEt ₃ Pb Et ₄ Pb	GFAAS	Packed column, 3% OV-101 on Chromosorb W	DL 40 pg as Pb	Gasoline and exhaust air laboratory study	49
Me ₄ Pb Me ₃ EtPb Me ₂ Et ₂ Pb MeEt ₃ Pb Et ₄ Pb	QFAAS	Packed column, 3% OV-101 on gas chromQ	DL 12–25 pg as Pb	Rainwater Motorway run off water Sheet dust Aerosol	50

Me ₂ Pb	QFAAS	Packed column, 10% OV-101 on Chromosorb W.	DL 10–270 pg as Pb	Environmental samples Laboratory studies	123
Me ₃ EtPb					
Me ₂ Et ₂ Pb					
MeEt ₃ Pb					
Et ₄ Pb					
Me ₃ PrPb					
Me ₂ Pr ₂ Pb					
Et ₃ PrPb					
Et ₂ Pr ₂ Pb					
Pr ₄ Pb					
Me ₃ BuPb					
Me ₂ Bu ₂ Pb					
Et ₃ BuPb					
Et ₂ Bu ₂ Pb					
Applications using GC/AED					
Me _x Sn ^{(4-<i>x</i>)⁺}	AED	HP-1: Capillary 25 m × 320 μm, 0.17 μm.	DL 0.05 pg as Sn	Water samples Sediment	104
n-Bu _x Sn ^{(4-<i>x</i>)⁺}					
Pr ₃ Sn ⁺					
n-Bu _x Sn ^{(4-<i>x</i>)⁺}	AED	Capillary column, 25 m × 0.255 mm i.d., XE-52XL with film thickness 0.25 μm	DL 6 pg as Sn	Sediment Fish	103

and, recently, biological tissues. In general, the early papers were only concerned with the trialkylated forms, which show high toxicity. However, the demand for understanding biogeochemical pathways and degradation patterns necessitated the refinement of analytical schemes to simultaneously determine other less substituted alkyltins. The best examples of this are in papers published by Müller. His first report [55] dealt only with the determination of tributyltin in environmental samples. Three years later, his analytical methodology [56] was extended to the simultaneous determination of 19 organotin species by capillary GC and FPD.

A result of this important technical evolution is the suspicion that new organotin species occur in the environment. Mixed butylmethyltin species have been reported by several authors. Maguire [57] first reported the occurrence of such compounds in sediments. Similar findings have been mentioned by several authors [58,59]. The question that new analytical developments need to answer is whether these results represent the exact distribution of organotin compounds present in the environment or whether the species could result from the sometimes drastic conditions applied to the sample extract during determination.

16.4.1.1. Analytical scheme

Organotin analyses are difficult because with one to three substituents the compounds are polar and non-volatile, reflecting their ionic character. Quantitative methods for their determination by gas chromatography include at least seven steps. The general scheme for organotin determinations from various matrices is performed in the following order:

- (a) acidification and conversion of alkyltin compounds (hydroxides, sulphides) to their respective chlorides and extraction into an organic polar solvent;
- (b) purification;
- (c) drying of the solvent (e.g. with CaCl_2);
- (d) derivatization by various methods to yield volatile tetrasubstituted species suitable for GC separation;
- (e) preconcentration by controlled evaporation of the solvent;
- (f) separation on a GC column;
- (g) detection by various types of detectors.

This analytical scheme is long and tedious to perform. The multiplicity of operations increases the chances of errors leading to poorer reproducibility. The exact order of some steps can be changed according to different authors. Some may add a second preconcentration step which can lead to improved detection limits. The importance of the clean-up step may vary from one method to another. Finally, an important source of error arises in the preconcentration steps during evaporation. Tetrasubstituted alkyltins are sometimes highly volatile, depending on the derivatization mode used and may result in important losses during evaporation of the solvent. Nonetheless, if applied carefully this general scheme allows the efficient determination of a wide range of long-alkyl-chain organotin compounds, in various kind of matrices, as a result of the important separation capabilities introduced with capillary columns. Sample preparation procedures can be used to distinguish between the different major trends developed. They can be summarized as follows:

- derivatization of organotin compounds by a Grignard reagent;
- conversion of organotins to their hydride derivatives;
- conversion of alkyltins to the chloride salts.

16.4.1.2. Derivatization by Grignard reactions

This analytical scheme involves the reaction with a Grignard reagent, in a solvent, to convert alkyltins ($R_xSnX_{(4-x)}$) into non-polar mixed tetra-alkyltins which are suitable for GC separation. This protocol has been applied successfully to the largest number of alkyltins in different matrices, such as waters, sediments and biological tissues.

After acid digestion of the sample, the organotins are extracted into a non-polar solvent. Commonly used solvents are benzene, pentane, hexane and dichloromethane. Acidification of the sample is usually performed with HCl. The addition of HBr has been shown to enhance the recovery of organotin species [52,54,59–61], probably by preventing adsorption on the walls of the container. The first extraction scheme with a solvent is most often sufficient for trisubstituted alkyltins but highly polar mono- and di- methyl or -butyltin compounds including ionic Sn^{4+} require complexation with fresh tropolone solution to extract these species efficiently into the solvent [54,56,60]. These conditions are not directly applicable to methylated tin species. More drastic acidic conditions using a combination of hydrobromic, hydrochloric, acetic and sulphuric acids in a high ionic strength medium are required [54].

Derivatization reactions have to be performed in an aprotic solvent and a drying stage is required prior to reaction. Peralkylation with a selected R' Grignard reagent (R' can be methyl, ethyl, butyl, pentyl or hexyl) in a solvent substitutes an alkyl group for the counterion of organotin compounds, to convert them into volatile tetraalkylated derivatives. The choice of the size of the alkyl group, R', depends both on the volatility of the compounds to be determined and on the type of analytical instrument used. High efficiency capillary gas chromatography allows derivatization with hexylmagnesium salts, to generate stable derivatives. The group R' can be methyl for the determination of butyltin [52,55] or phenyltin compounds [61]. Butylation has also been used for the determination of methyltin species in the environment [54]. Methylation or butylation have been abandoned, because mixed methylbutyltin species have themselves been suspected to occur in the environment. Derivatized compounds should have very different and higher boiling points than the solvent, to allow the preconcentration by evaporation. Ethylation [56] and pentylation [59,62–65] have been successfully applied to the determination of organotin compounds in the environment. Finally, hexylmagnesium bromide was used to improve the separation between butyltin species and to minimize losses during sample concentration [66–68]. Hexylalkyltins are also more thermally stable, facilitating the later mass spectral determination. However, several precautions should be taken to ensure optimal determinations during this long analytical procedure. An internal standard is usually added at the beginning of the scheme to establish the efficiency of the protocol. One should use more than one internal standard with a large difference in boiling points if several alkyltin species are to be determined. Differential evaporation during the preconcentration steps could lead to errors in quantification [28]. Tropolone should be used in the

dark, since dismutation of trimethyltin to tetramethyltin in the presence of light was reported [69].

New developments in the sample-preparation stage allow simpler and faster methods for the determination of alkyltins. Liquid-liquid extraction should soon be replaced by simultaneous liquid-solid partitioning and preconcentration. To simplify parts of the analytical protocol, the liquid-liquid extraction and preconcentration steps have been substituted by liquid-solid extraction. Müller first used an apolar resin (Bio-Beads SX-2) to remove tributyltin bromide from aqueous solutions and obtained good recoveries [55]. Organotins were later extracted as chlorides from tap water using a tropolone- C_{18} silica cartridge [56]. Important progress is to be expected with these solid extraction/preconcentration procedures in the future.

The general advantages of this analytical protocol are linked to the fact that Grignard derivatization yields stable tetrasubstitutes with low evaporative losses when using alkylating reagents with the higher alkyl- or arylgroups. Polar clean-up procedures are also less likely to simultaneously extract the non-polar derivatives [70].

16.4.1.3. Hydride generation

Derivatization by hydride generation is also widely used for the determination of alkyltins. Two general approaches have simultaneously been developed and adapted for gas chromatography based instrumentation. The first uses extraction of analytes by direct hydride generation from an acidic digest of the sample, then trapping of the hydrides and separation by gas chromatography. Like these techniques, it is most efficient with small alkylated compounds (methyl- and butyltins). Applications have mostly focused on determination of methyl- and butyltin compounds in water and sediment matrices. Trapping of the hydrides can either be done cryogenically [71] or at room temperature on Tenax, using a commercial purge-and-trap system [72,73]. The detectors used are either classical FPD or mass spectrometry [74]. These purge-and-trap techniques have revealed the occurrence of volatile methyltin hydrides in the environment [75].

The second approach is also based on liquid/liquid extraction of organotin compounds from the sample, but the derivatization is then performed with borohydride in an aqueous medium or in another solvent [76–81]. The organotin hydrides are similarly concentrated by slow evaporation of the solvent and injected into a gas chromatograph for separation and detection. This procedure has mainly been applied to di- and trialkylated compounds such as the butyl- and phenyltins. It has been applied to water and sediment samples but most of the applications reported have been developed for analysis of biological tissues. All techniques include an important clean-up step which is usually accomplished by passing the extracted compounds in the solvent (preferably hexane to prevent water deactivation of the cartridge) through a silica microcolumn. Since most of these techniques are focused towards the determination of organotin compounds in biological matrices, the products of the first extraction procedures yield high levels of lipids. Direct determination of organotins can be performed after this first extraction but the high lipid content will degrade the column, generating erratic reproducibility, and prevent good concentration of the solvent, thereby leading to poor overall detection limits. The clean-up procedure removes more than 90% of the lipid content of the sample but does not eliminate pigments

or other extraneous material [80]. Analyte recoveries should be carefully checked, since in the case of Ph_3SnCl , 75.9% was obtained for a $10\text{ }\mu\text{g/g}$ concentration whereas it was only 33% for a $0.1\text{ }\mu\text{g/g}$ concentration [78]. Despite this problem, the clean-up stage has generally been shown to increase the sensitivity of the analysis, and to improve tailing of the chromatographic peaks by drastically reducing the occurrence of interfering compounds. Several types of commercial silica gel cartridges have been tried and all gave satisfactory results.

As with the analytical protocols using alkylation via Grignard reactions, many authors have tried to eliminate some steps. Extraction and preconcentration of tributyltin compounds from estuarine waters has been achieved using a bonded C_{18} solid phase adsorbant [82] prior to hydride derivatization.

The simultaneous extraction into dichloromethane and hydritization by NaBH_4 of butyl- and methylbutyltin species was shown to yield excellent recoveries of these compounds from natural water samples [39,58]. This method can process large sample volumes (800–1000 ml) and thus gives good detection limits, expressed on a concentration basis [83].

A new trend is also appearing in the literature. On-column hydride generation allows the direct injection of a solution of butyltin chloride into the gas chromatograph [80,84]. Derivatization of the extracts is performed directly at the top of the column either on solid NaBH_4 pellets introduced at the entrance of the chromatographic column [84] or via a packed reactor placed in the injection port of the gas chromatograph [80].

An alternative to the classical hydrogenation step using NaBH_4 in water is presented by the use of NaBEt_4 to form ethylate organotin species. This method was introduced for the determination of alkylleads and alkylmercury compounds by cryogenic trapping, as mentioned earlier in this chapter [8] and has been successfully applied to the determination of organotin compounds in water [85,86] (Fig. 16.6) and sediments [28]. In contrast to the hydride technique, this method does not appear to suffer from interferences present in sediment extracts.

16.4.1.4. Determination of the chloride salts

Since the electron capture detector is sensitive to both trialkyltin, dialkyltin [87–89], triphenyltins [90], and to tricyclohexyltin chlorides [91], there have been several developments which allow quantitative data to be obtained from biological material. In general, the detection limits obtained with the electron capture detector are poor. In comparison to methods using Grignard reagents, these methods are limited in their detection limits and by the range of alkyltin species determined in one sample. Despite the limitation mentioned above, some of the interest in the detection of organotins as their chloride salts relates to the possible direct concentration of trialkylated compounds, “in the field”, using a solid phase adsorbant. In estuarine and marine waters, where toxicity problems are most acute, there is a high probability of the occurrence of these compounds as chloride salts. Their direct extraction by adsorption/preconcentration on a C_{18} bonded column, then gas chromatographic separation, and detection by ECD suppresses many analytical steps in the determination protocol [92]. This also combines sample preconcentration and may facilitate storage problems. The sampling and preconcentration step can also be performed

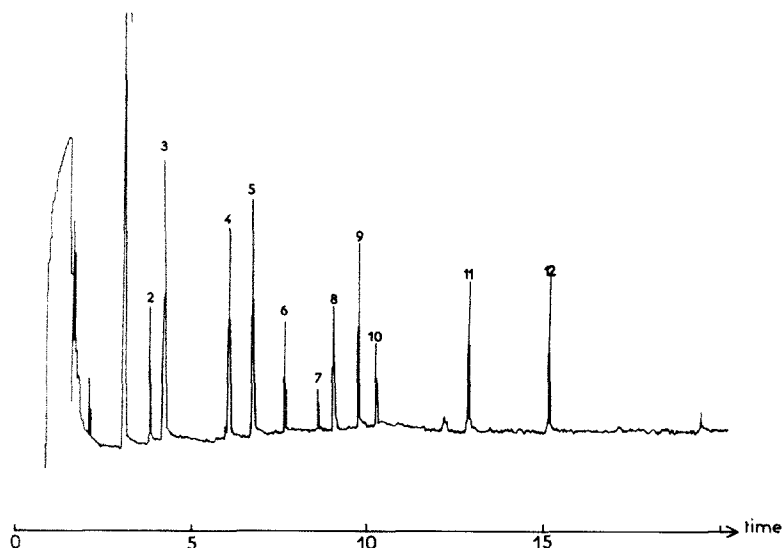


Fig. 16.6. Chromatograms of organotin compounds after simultaneous aqueous extraction derivatization by NaBEt_4 by capillary gas chromatography and flame photometric detection (initial compounds prior ethylation reaction). 1, Methyltin trichloride; 2, methylbutyltin dibromide; 3, tetraethyltin; 4, butyltin trichloride; 5, tri-*n*-propyltin chloride; 6, dibutyltin dichloride; 7, phenyltin trichloride; 8, tributyltin chloride; 9, octyltin trichloride; 10, tetrabutyltin; 11, dodecyltin trichloride; 12, triphenyltin hydroxide. All compounds 50 pg as Sn; after [86].

simultaneously with the use of an in situ sampler. A time-integrating, remotely moored automatic sampling and concentration device allows direct concentration of butyltins on an octadecyl (C_{18}) bonded reversed-phase solid sorbant [93]. This approach looks promising but redistribution of the compounds has been reported. Highly acidic, non-hydrogen bridged Si-OH groups, found in the silicate sorbant phases may result in important misidentification of the initial alkyltin species distribution in water samples.

16.4.1.5. Detectors

Several types of detector have been used for the determination of alkyltin in environmental matrices. Their technical specificities have closely controlled their extension and possibilities and the mode of preparation of the analytes. One of the first classical GC detectors was the electron capture detector (ECD). Other popular detectors in increasing order of specificity are the flame photometric detector (FPD) and the use of atomic absorption with atomization in an electrothermal quartz furnace. Other detectors are being used, among them mass spectrometry. Environmental analyses by gas chromatography and mass spectrometric detection were developed relatively early [52] but were not very sensitive. Considerable improvements in sensitivity have been obtained by coupling cryogenic trapping before the chromatographic stage, which allows the detection of picograms of alkyltins in various matrices [74].

The ECD detector has been shown to be efficient for the detection of organotin as chlorides, but is less sensitive and specific than the other detectors mentioned previously. The flame photometric detector is certainly now the most popular for organotin determination. However, tin is difficult to excite thermally in flames and several important developments and modifications have been made to conventional FPD for alkyltin determination [86,94–97]. Quantitation is based on monitoring the red fluorescence molecular emission of the Sn–H species at 609.5 nm. This kind of detector yields excellent sensitivity [56,98]. The best overall sensitivity is obtained with a filterless FPD, but then the detector lacks specificity [98]. To improve the selectivity, an additional scan can be performed in the broad and less sensitive 360–490 nm blue region (SnO band) [96].

Interference in the detection stage have been mentioned with various kinds of detectors. The most common interferences reported for the FPD detectors are generated by the presence of sulphur compounds, hydrocarbons and germanium species [18,71,99]. The most specific detector so far is atomic absorption with an electrothermally heated quartz furnace (QFAAS). Many couplings with gas chromatograph instruments have been mentioned. This detector is generally highly selective for tin but it can also suffer from interferences generated by the presence of hydrocarbons [100]. In general, good agreement is obtained between results obtained with detectors such as QFAAS and FPD [101]. Some references mention the use of a promising detector for the determination of organotin compounds based on the fact that in the vapour phase they have been found to quench ionization in a hydrogen-atmosphere flame [102]. When this system is used as a chromatographic detector, the organotin compounds produce negative peaks while hydrocarbon compounds produce a positive response that is 105 times less sensitive than that of the organotin. The sensitivity of this detector is equivalent to that of a classical flame ionization detector (FID) but its tremendous advantage is its selectivity against hydrocarbons [99].

Recently, other types of detector have been used for the determination of organotin compounds after CGC separation. MIP/AED for organotin is gaining increasing popularity owing to its selectivity and high sensitivity [103,104]. These techniques feature extremely low absolute detection limits, estimated as 0.05 pg of Sn [104] and will certainly contribute to our understanding of the bio-geochemical pathways of tin in the environment. There is no doubt that the future will see hyphenation between supercritical fluid chromatography (SCF) directly interfaced with MIP/AED detectors [105]. ICP/MS detectors have also been interfaced with gas chromatography for the determination of tin compounds [106]. Suyani and co-workers [107] have interfaced a helium MIP cavity to the MS detector of ICP/MS after gas chromatography separation. These expensive solutions allow ever-lower levels of simultaneous detection for elements and the ability to obtain isotope ratio information.

16.4.2. Organolead compounds determination

Organolead compounds form another class of organometallics which have been massively introduced into the environment. Most sources originate from tetraalkylleads (R_4Pb) used in petrol additives as antiknocking agents. Their wide dispersion in the environment and subsequent degradation give rise to a wide variety of methylated, ethylated

and mixed ethylmethyl-lead species. The tri- and dialkyl-lead compounds are fairly persistent species and are ubiquitous in the environment [108]. A wide array of techniques has been developed and all are very similar to those applied to organotin compounds. Alkyllead determination has certainly been attempted for the widest array of matrices, such as air, water (river, estuarine, rain and tapwater), sediments, biological tissues [109], and even snow samples [110].

16.4.2.1. Extraction and derivatization

The extraction and derivatization procedures are quite similar to those applied with organotins. The R_4Pb compounds are simply extracted directly from environmental samples by a variety of organic solvents such as hexane or benzene. Derivatization of the ionic (R_2Pb^{2+} and R_3Pb^+) species to produce volatile tetraalkylleads has been shown to be necessary for environmental analysis. Ionic alkylleads (R_2Pb^{2+} and R_3Pb^+) can be extracted and preconcentrated from environmental water samples in the presence of NaCl and NaDDTC in benzene [111], *n*-hexane [109] or pentane [112]. Sample extracts are then concentrated by gentle evaporation prior to derivatization by either *n*-butylmagnesium chloride [112–115], *n*-propylmagnesium chloride [113], or phenylmagnesium chloride [114]. As is observed for organotin compounds, recoveries of R_3Pb^+ are always high whereas some discrepancies may be observed with R_2Pb^{2+} [109]. Water sample volumes usually range from 0.5 to 10 l. Smaller volumes may be used (0.1 l) when the determination is performed with a sensitive system such as HRCGC-MIP/AES [116]. Lobinski and Adams have compared two derivatization reactions applying either propylation or butylation for the speciation of alkylleads in rain and tap water at the $pg\ l^{-1}$ level. They report similar results except for finding higher detection levels of diethylated lead species when butylation is employed [116].

The R_4Pb species can be determined in sediments and biological tissues. Chau and co-workers [117] extracted 2 g of homogenized fish tissues or 5 g of wet sediment with 5 ml of 0.1 M EDTA and 5 ml of hexane in capped test-tubes. The mixture was centrifuged and a 5–10 μ l aliquot of the extract was injected into a GC/AAS apparatus. For the di- and trialkylated species, a wide variety of chemical extraction and reagent combinations

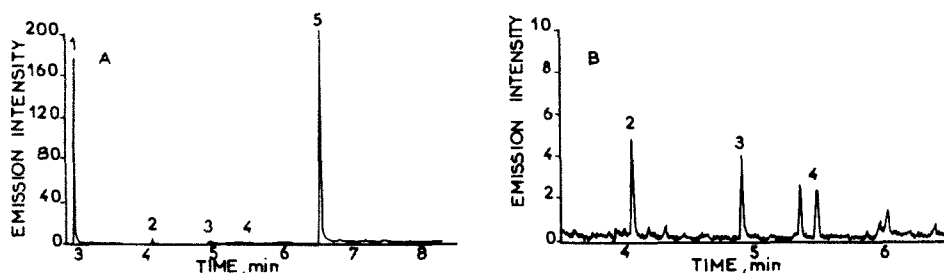


Fig. 16.7. Chromatogram of an extract of rain water sample derivatized by propylation (after preconcentration). (1) Me_3Pb^+ ; (2) Me_2Pb^{2+} ; (3) Et_3Pb^+ ; (4) Et_2Pb^{2+} ; (5) Pb^{2+} (reproduced from [116] with permission from Elsevier).

exists for the recovery of R_3Pb^+ and R_2Pb^{2+} from sediments and biological tissues. A typical sequence of operation is as follows, for a biological tissue [118]. After complete digestion of 2 g of fish tissue by 5 ml of tetramethylammonium hydroxide (TMAH), the solution is extracted with 3 ml of benzene with continuous agitation for 2 h in the presence of 2 g of NaCl and 3 ml of 0.5 M NADDTC. The phases are separated by centrifugation and 1 ml of the extract is butylated by addition of 0.2 ml of 0.9 M *n*-butylmagnesium chloride in tetrahydrofuran. The mixture is then washed with 2 ml of 0.5 M sulphuric acid and the phases are separated. The final extract is dried with anhydrous sodium sulphate and 10 μ l is finally injected into the GC/AAS. This procedure allowed the authors to report the simultaneous measurement of R_4Pb , R_3Pb^+ and R_2Pb^{2+} in a fish sample [118].

Other derivatization solutions have been attempted but do not appear to be appropriate for the determination of alkylleads in environmental samples. Hydride generation of lead species from human urine samples was not completely satisfactory because of the poor stability of lead hydrides and poor reproducibility [119].

16.4.2.2. Detectors

As mentioned earlier in this chapter, one of the first hyphenated systems was realized for the determination of alkylleads in petrol additives, using with a packed-column gas chromatograph directly connected to the flame burner of an AAS [32]. Graphite furnace atomizers have been used with AAS detection [109]. Other detectors such as AFS and ICP/AES have also been applied to detect the lead present in the effluents of the gas chromatograph. However, most popular solutions rely on the hyphenation between a gas chromatograph and atomization in an electrothermally heated quartz cell aligned in the beam of an AAS. Generally, the simple interface consists of a stainless steel tube between the GC column and the quartz atomizer. In this case also, the transfer-line temperature has been shown to significantly influence the sensitivity for compounds requiring a high elution temperature [116].

The introduction of hydrogen to the atomization tube also improves significantly the performance of the instrumentation [113,120]. Excellent sensitivity is generally obtained with absolute detection limits between 40 and 90 pg (as Pb) [121]. Here again, capillary gas chromatography using microwave-induced plasma atomic emission spectrometry provides the most sensitive solution reported to date, with absolute limits of detection ranging from 0.02 to 0.1 pg as Pb [116] (Fig. 16.7).

Finally R_3Pb^+ can be separated directly by GC, with detection using either an electron capture (ECD) [122] or flame ionization detector (FID) [123], but the resulting sensitivity of the whole protocol is inadequate for environmental analysis.

16.4.3. Organomercury compounds determination

Mercury is certainly one of the contaminants which is best known because of the occurrence and high toxicity of methylmercury, $MeHg^+$. This is considered to be one of the most dangerous pollutants present in the environment. Concerns arise specifically since mercury accumulates in the food chain, principally as $MeHg^+$ [124]. Considerable efforts

have been made to determine MeHg^+ principally in the environment, in air, water, sediment and fish samples. Most chromatographic applications have been focused on the determination of MeHg^+ in biological tissues. However, despite the important efforts made, it is worthwhile mentioning that in comparison to other organometallic species, the method developments related to mercury speciation are not completely satisfactory and most of them are cumbersome to apply.

16.4.3.1. Sample preparation and chromatography

Traditionally, gas chromatographic analysis of organomercury compounds has been performed with packed columns and electron capture detection (ECD). The classic method for extracting and separating organomercurials from organic matrices for ECD detection is well established and commonly known as the Westöb method [125,126], focusing on the determination of MeHg^+ . Most of the protocols used to date are derived from this methods and the general scheme can be summarized as follows:

- (1) methylmercury is liberated from its protein bond by displacing the mercapto group with halogen ion at low pH;
- (2) a selective extraction of the organomercury species is performed in an organic solvent (most frequently toluene);
- (3) the organic extract is purified from interfering impurities by extraction in an aqueous solution in the presence of a thiol compound (cysteine);
- (4) redissociation of the organomercury-thiol complex, as in the initial step;
- (5) re-extraction into an organic solvent and eventual preconcentration by gentle evaporation of the solvent;
- (6) injection into a GC-ECD apparatus.

A recent and slightly different version of this procedure replaces the cleaning step by repeated washing of the sample with acetone and toluene [127].

The chromatographic step of this procedure needs to be carefully controlled since the mercury-halide bond exhibits a very polar character and interacts strongly with the column, leading to severe tailing of the chromatographic peaks. To overcome this problem, "passivation" of the column prior to determination, using a concentrated solution of mercury chloride in a solvent (benzene or toluene) has been proposed [127,128]. The chromatographic behaviour and interaction of organomercury halides are still problems which are under investigation by numerous research groups. The resolution potential of capillary columns versus classical packed columns is still controversial. The first tests of wide bore thick-film fused silica open tubular (FSOT) columns (0.53 mm i.d., 1.2 μm film thickness) were reported to be very successful [129,130]. The efficiencies of high- and-low polarity semi-capillary columns (SGE BP-1 (methylsilicone), BP-5 (phenylsilicone) and BP-20 (polyethyleneglycol) columns, 25 m long, 0.5 and 1 μm film) have also recently been investigated [131]. In all cases, conditioning was necessary, but all gave satisfactory results and the use of a low polarity column for routine analysis was recommended. CP-Sil 8 CB capillary columns (wide bore and 5.35 μm thick stationary phase) were also found to be satisfactory [132]. A comparison of packed versus capillary columns (Superox-FA FSOT) has also been performed recently for the determination of alkylmercury by head space analysis using either ECD or MIP/AES detectors. The col-

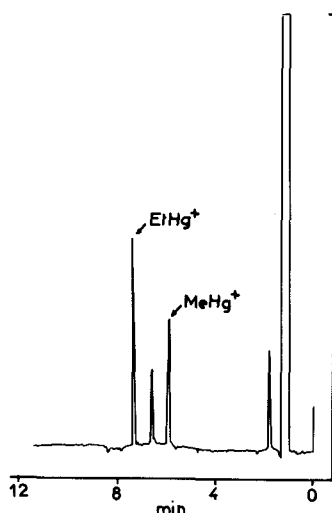


Fig. 16.8. Chromatograph from the analysis of MeHg^+ in an extract from an homogenized fish sample (reproduced from [134] with permission from Springer-Verlag).

umns need to be water-resistant. In this study, there was no clear advantages in the capillary columns over traditionally packed columns [133].

In order to avoid the chromatographic problems, several alternative approaches have been used. Alkylmercurials can be converted into their bromide derivatives by the addition of cupric bromide at the clean-up stage. Determination by capillary gas chromatography (OV-275) with ECD detection yields excellent chromatographic resolution for EtHg^+ and MeHg^+ (Fig. 16.8) [134]. Finally, to eliminate the problem generated by the polar mercury-halide bond, the mercury species can be butylated by a Grignard reagent, a procedure similar to that described for Sn or Pb compounds, to yield non-polar dialkyl derivatives [135]. As the electron-capturing halide moiety is absent from these derivatives, mercury specific detection is necessary and is achieved by MIP/AES [135].

16.4.3.2. Detectors

The non-specificity of the ECD detector and the possibility of co-elution with other compounds have triggered the interest in interfacing chromatography systems to Hg-specific detectors. The volatility of Hg^0 and the ease of thermal decomposition of alkylmercury compounds have allowed a wide variety of solutions which use cold vapour atomic absorption determination in a fused silica quartz cell. Various methods for the reduction of mercury have been proposed. After chromatographic separation, dialkylmercury compounds were decomposed to give Hg^0 after passing through a flame ionization detector and detected in small cold vapor unit as detector MAS 50 [136]. Using a simple GC/AAS interface heated to 600°C , thermal decomposition of alkylmercury species (Me_2Hg , Et_2Hg , MeHgCl and EtHgCl) was obtained in the heated capillary line just prior to entry

into the AAS [137,138]. Atomization of organomercurials is achieved in the FPD and subsequent detection by AAS did not yield good sensitivity, but produced pronounced tailing of the chromatographic peaks. Significant improvements were observed when the atomization and detection were performed directly in the fused silica furnace held at 780°C using an oxygen flow and after considerable reduction of the transfer lines [139]. A recent and sensitive set-up applied to the determination of MeHg^+ in biological tissues, hyphenates detection using the ECD with direct confirmation of Hg in the peak detected by on-line thermal decomposition (900°C) in a quartz tube, cooling, preconcentration on gold wool and final detection of the cold vapour in an AAS [140].

Despite the different combinations which use AAS as a detector, the most frequent solution adopted for the determination of organomercurials after GC separation is certainly MIP/AES, because it avoids the predecomposition step required in the AAS detection mode. The first applications of the MIP/AES detector for mercury speciation and detection were reported in the 1970s [141–143]. It has been applied since by numerous authors to the determination of alkylmercury in water, sediments [144–147] and in biological samples [148]. Headspace analysis using GC-MIP/AES has also been reported for the determination of MeHg^+ in fish samples [149]. This approach has recently been improved [150,151].

However, despite the final sensitivities of the detectors, most of the above methods require large sample volumes, tedious solvent extraction procedures, and usually lead to the final determination of only the MeHg^+ species. Considerable improvements are expected in this area, as mentioned earlier in this chapter. The recent description of the feasibility of quantitative in situ aqueous ethylation of ionic mercury and methylmercury ion, followed by on-line preconcentration and detection by AFS or AAS will certainly produce a wealth of information since it allows all mercury species to be detected in the same chro-

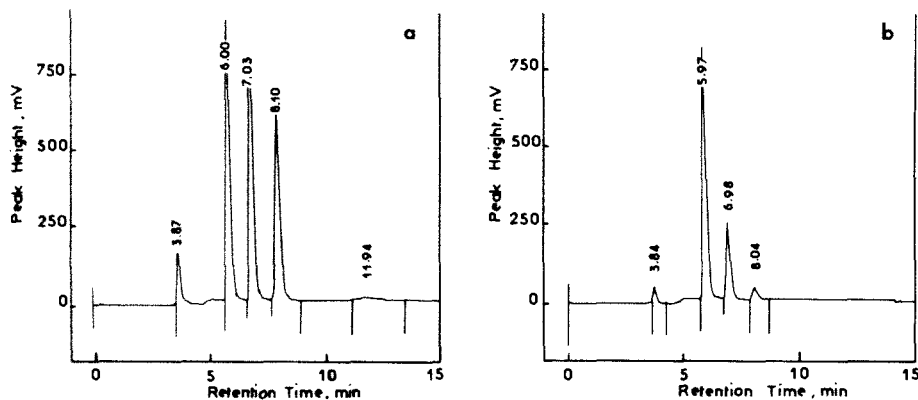


Fig. 16.9. Typical chromatograms of organomercury species obtained by aqueous phase ethylation. (a) Tap-water sample containing spikes of 454 pg Hg as $(\text{CH}_3)_2\text{Hg}$ (6.00 min peak), 429 pg as Hg CH_3HgCl (7.03 min peak; methylethylmercury) and 400 pg Hg as HgCl_2 (8.10 min peak; diethylmercury). Peak at 3.87 is residual Hg^0 . (b) Fish tissue digestate, equivalent to about 1 mg *Catostomus commersoni*, spiked with 413 pg Hg as $(\text{CH}_3)_2\text{Hg}$ dimethylmercury (reproduced from [25] with permission from NRCC Canada).

matographic run (Fig. 16.9). This approach still needs significant refinement, but its simplicity and sensitivity will certainly supplant the traditional techniques which use solvent extraction based methods and GC-AAS or GC-MIP/AES techniques.

16.5. SEPARATION BY LIQUID CHROMATOGRAPHY

Liquid chromatography (LC) is another popular method for the separation of organometallic species prior to detection. The use of LC, and in particular, high performance liquid chromatography (HPLC), considerably increases the number of chemical and physical species which can be studied. Separation with HPLC offers several advantages over gas chromatography (GC). One of the major advantages is that the analyte can be separated at ambient temperature with no need for derivatization. This not only reduces the sample through-put time but also reduces possible losses during the processes. Furthermore, there are more variable operational parameters; both the stationary and mobile phase can be varied simultaneously to achieve better separation. A large variety of stationary phases is available and gel-permeation, adsorption, ion-exchange, normal- and reverse-phase chromatography allow the separation of ions, volatile species and high-molecular-weight organometallics, as well as large complex biological species. The technique has better overall versatility than GC separations.

For the separation of analytes of metallic or organometallic species, ion-exchange chromatography is the most widely applied form of HPLC, because of its ability to separate free and complex ion species. Reversed phase techniques are also widely utilized with the use of an ion-pairing agent.

In HPLC operation there is a continuous liquid flow, typically in the range of 0.05–6.0 ml min⁻¹ of the mobile phase. The detector system must be compatible with this flow. In the flame mode, continuously operating atomic absorption spectrometry (AAS) and atomic emission spectroscopy (AES) are compatible with the effluent flow of the HPLC system. The effluent can easily be introduced into the nebulizer of a flame AAS (FAAS) instrument. An important factor in interfacing the HPLC with an AAS detector is the dispersion, not only in the column but especially in the interface tube and the AAS detector. It is clear that dispersion not only reduces the element sensitivity but can also destroy the separation originally obtained in the column.

FAAS was the first candidate for coupling with liquid chromatography but the coupling gave a low sensitivity from a weakly sensitive detector in combination with a low analyte transport efficiency (5–10%). Many research groups then shifted to graphite furnace (GF) AAS because of its excellent detection limits. However, a drawback in interfacing HPLC with GFAAS lies in the incompatibility of the continuous elution of the mobile phase with the discontinuous nature of the GFAAS.

Coupling of LC to inductively coupled plasma (ICP) systems hardly improves the detection limits when atomic emission spectroscopy (AES) is used. However, when HPLC is coupled to ICP/MS (mass spectrometry), a rather sensitive multi-element detection system is obtained (Fig. 16.1). The coupling of liquid chromatography with atomic spectrometry has been reviewed in several papers [152–159] and books [5,160,161].

16.5.1. High performance liquid chromatography interfaced to flame atomic spectrometry (HPLC-FAAS)

16.5.1.1. Continuous interfaces

The introduction of the eluate from a liquid chromatograph into an AAS detector is not yet as well developed as for the gaseous eluate from a GC. Common flow rates in LC are typically in the range of 2–6 ml/min; the uptake rate of a flame AAS nebulizer is in the same range so it is possible to couple the two techniques directly using an interface tube. However, the main difficulty in interfacing HPLC and AAS is in balancing the flows because the optimal flow rates in HPLC are set by chromatographic separation criteria while the nebulizer uptake rate is set using the maximum sensitivity for the aspiration of standard solutions. Most flow rates for the nebulizer are higher than HPLC flow rates, so the nebulizer is starved of liquid. To overcome this problem of starvation, an additional solvent reservoir can be used at the end of the column [162], but this will lead to undesirable sample dilution. Another possibility is to attach a Teflon funnel to the nebulizer [163]. Introducing a small T-piece into the transfer line also prevents starvation of the nebulizer [164–166]. The effluent droplets from the column exit were caught in the funnel and nebulized one at a time. The chromatogram consisted of a series of spikes but there was no loss in sensitivity because 100 μ l droplets were sufficient to give a steady state signal. Only a few applications of the analysis of organometallics using HPLC directly coupled to FAAS have been reported [40,166–172], because of an inadequate overall sensitivity for environmental analysis (Table 16.3).

Some of these applications deal with the analysis of environmental samples [40,166,171] the analysis of tributyltin TBT in seawater and of alkyllead compounds in water, soil and sediments. Directly coupled FAAS utilizing pulse nebulization and a slotted tube atom trap gave a detection limit for TBT (as Sn), after an 800-fold concentration step, of 0.47 μ g l⁻¹ in seawater [166]. When a long absorption tube (1 m \times 8 mm i.d.) is used after a total consumption burner detection limits for TBT of 0.03 μ g l⁻¹ (as Sn) in seawater can be achieved after a 1000 fold concentration step [40].

Since the analyte transport efficiency of conventional FAAS nebulizers is in the range of 5–10% [173], significant improvements can be gained by improving the efficiency of the nebulizer system. The aerosol chamber from a nebulizer system originally developed for inductively coupled plasma [174] has been adapted for a nebulizer interface in FAAS, and results in an almost 100% analyte transport efficiency as a result of improved flow characteristics and efficient desolvation [167,174]. The design includes a heating zone, an auxiliary gas connection and a conical inlet for the aerosol. Some of the oxidant gas is used as nebulizing gas, and the remainder is added to the auxiliary gas flow. With this design, the detector signal was ten times better than that obtained using a conventional nebulizer. Analysis of TBT gave a sensitivity improved by a factor of four.

Recent developments in direct HPLC-furnace interfaces include a thermospray micro-atomizer heated in a flame (1400–1600°C), where the HPLC effluent is flash evaporated to an aerosol before entering the furnace [171]. Detection limits for four ionic alkyllead compounds are significantly improved and are in the range of 1–2 ng. For sediments, this

means that sub-ng g⁻¹ alkyllead determinations for 10 g of sediment are feasible. A summary of several applications is given in Table 16.3.

16.5.1.2. On-line postcolumn reactions in HPLC-FAAS

The interface which involves the use of a hydride generator for postcolumn hydride generation results in continuous, real-time signals. After HPLC separation, it converts alkylmetal species into their corresponding hydrides, which are then passed to a quartz tube in the AAS. The hydride generation unit not only converts the analyte to volatile forms, but also isolates them from the sample stream minimizing later interferences. This technique, however, although it generates continuous signals, is limited to organometals which can form volatile hydrides.

A schematic diagram of the chemical hydride generator is given Fig. 16.10. After HPLC separation, the eluent is introduced into the hydride generator, and mixed with hydrochloric acid and then with a 1–4% NaBH₄ solution. The gaseous hydrides formed in the reaction coil are separated in a gas-liquid separator and introduced by inert gas flow into the heated quartz absorption cell of a FAAS. The quartz cell has been heated by flames or by electrical heating. Ethylation with tetraethylborate solution can also give volatile derivatives [175]. Postcolumn ethylation has been successfully used in the analysis of ionic alkyllead compounds. The absolute detection limit was 0.1 ng Pb, which is comparable to the general sensitivity of the GC furnace AAS.

The most popular use of the postcolumn hydride generator has been for the determination of reducible arsenic species [164,176–179]. These can first be preconcentrated onto an anion-exchange column and then eluted, by changing the mobile phase, to the separation column. In this way, improved detection limits and removal of matrix interferences can be accomplished. The analysis time can be very short; less than 10 min [164,176,177,179] or even less than 3 min [178]. With these approaches, arsenic species have been determined in a wide range of samples, such as pore waters, estuarine waters, urine, fruit and vegetables [180].

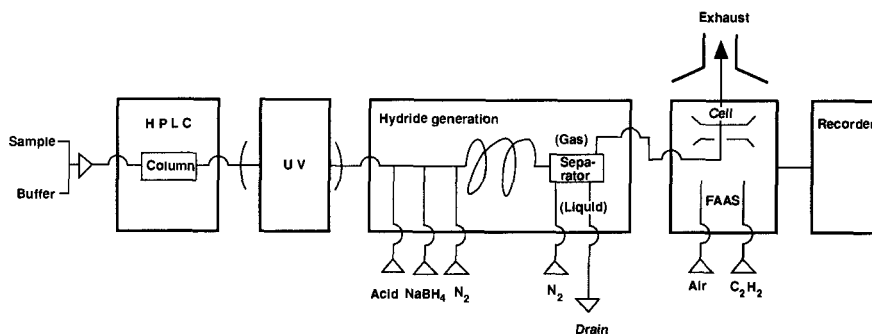


Fig. 16.10. Schematic diagram of HPLC-HG-FAAS system. HPLC Model 5000 high performance liquid chromatograph (Varian); hydride generation = VGA-76 hydride generation apparatus (Varian); FAAS = AA-775 flame atomic absorption spectrometer (Varian) (reproduced from [179] with permission from Elsevier).

TABLE 16.3

APPLICATIONS OF HYPHENATED SYSTEMS USING LIQUID CHROMATOGRAPHY COUPLED TO ATOMIC SPECTROMETRY

Element	Detector	Chromatography	Comments	Sample	Ref.
Applications using HPLC-FAAS					
Sn	Flame AAS using $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame	ODS Spherisorb S5W, 250×3.0 mm i.d. Eluent: acetone/pentane (3 + 2) at 1.0 ml min^{-1} for methyltin compounds; acetone/pentane (7 + 3) at 1.2 ml min^{-1} for ethyltin compounds	Linear up to $50 \mu\text{g}$ DL (2 σ) 11–19 ng Sn Injection $50 \mu\text{l}$	Standard solution of methyl and ethyl tin compounds	168
Pb	Flame AAS using an air- C_2H_2 flame	$\mu\text{Bondapak C}_{18}$ column Eluent: acetonitrile-water (7 + 3) at 3 ml min^{-1}	Linear from 1.1 to $11 \mu\text{g}$ of total lead DL 10 ng $20 \mu\text{l}$ injection	Tetraalkyllead compounds in gasoline	170
Sn	Flame AAS using pulse nebulization and a slotted tube atom trap	Partisil SCX-10, 250×4.6 mm i.d. Eluent: methanol/water in 0.1 M NH_4OAc (80:20) at 3 ml min^{-1}	Chloroform extraction of TBT from seawater, transfer to methanol DL (2 σ) 200 ng Injection up to 2 ml	Tributyltin in seawater	166
Sn	Flame AAS with a concentric glass nebulizer mounted in a heated aerosol chamber	Partisil SCX-10, 250×4.6 mm i.d. Eluent: methanol/water in 0.1 M NH_4OAc (80:20) at 2 ml min^{-1}	Five-fold dilution in dichloromethane Linear range up to $40 \mu\text{g}$ DL (3 σ) 100 ng Injection $200 \mu\text{l}$	TBT and DBT in wood preservatives	167
Sn	Flame AAS Total consumption burner Absorption tube $1 \text{ m} \times 8 \text{ mm}$ i.d.	ODS-80 TM, 250×4.6 mm i.d. Eluent: THF/water/AcOH (54 + 38 + 8) containing 0.2% m/v tropolone at 0.9 ml min^{-1}	Linear from 5 to 400 ng Sn DL (3 σ) 5 ng Injection $200 \mu\text{l}$	Eight organotin compounds in seawater	169

Pb	Flame AAS using a thermospray interface and a microatomiser $T = 1400\text{--}1600^\circ\text{C}$	Nucleosil C ₁₈ or C ₁₈ Bondapak guard column with Hypersil ODS or Nucleosil C ₁₈ analytical column, 150×4.6 mm i.d. Eluent for hexane extracts: methanol/water (3 + 1) containing 0.6 mg ml ⁻¹ ammoniumtetramethylenedithio carbamate	Mean recovery for five alkylleads $81 \pm 18\%$ DL 0.8–1.6 ng Injection 50 μ l	Ionic methyl- and ethyl-lead in spiked water, soil and sediment samples Extraction with a mixture of hexane and dimethyldithio- carbamate Concentration to 0.2 ml	171
Applications of HPLC-FAAS using post column reactions					
Sn	Flame AAS using continuous hydride generation and electrothermal quartz tube atomization $T = 950^\circ\text{C}$	ODS Spherisorb S5W, 250×3.0 mm i.d. Eluent: acetone/pentane (3 + 2) at 1.0 ml min ⁻¹ for methyltin compounds; acetone/pentane (7 + 3) at 1.2 ml min ⁻¹ for ethyltin compounds	The design of a miniature hydride generator is given Linearity up to 100 ng DL (2 σ) 8–14 pg Sn Injection 50 μ l	Standard solutions of methyl- and ethyl-tin compounds	168
As	Flame AAS using continuous hydride generation and electrothermal quartz tube atomization $T = 800^\circ\text{C}$	Dionex anion-exchange 500×3 mm Eluent: 0.0024 M NaHCO ₃ /0.0019 M Na ₂ CO ₃ /0.001 M Na ₂ B ₄ O ₇ at 2.6 ml min ⁻¹	Miniature hydride generator Linear up to 80 ng ml ⁻¹ DL (2 σ) 3–16 ng ml ⁻¹ Injection 800 μ l.	Speciation of As ^V , MMA and <i>p</i> - aminophenyl arsonate in synthetic air samples	177

TABLE 16.3 (continued)

Element	Detector	Chromatography	Comments	Sample	Ref.
As	Flame AAS with a silica tube heated by an air-acetylene flame Continuous hydride generation	Zipax ion-exchange column, 100 × 5 mm i.d. guard column in series with a BAX-10 ion-exchange column, 200 × 5 mm i.d. Eluent: 0.1 M ammonium carbonate at 4 ml min ⁻¹	Linear from 3-15 ng As. D.L. (2σ) 2 ng for As(III) and 1 ng for As(V), MMA and DMA. Sensitivity dependent on the G-L separator design. Injection 100 μl.	Speciation of As ^{III} , As ^V , MMA and DMA in soil water and commercial bottled waters	178
Sn	Flame AAS with a borosilicate glass tube Continuous hydride generation	Partisil-10-SCX column, 250 × 4.6 mm i.d. Eluent: methanol/0.1 M ammonium acetate (80:20)	UV decomposition of TBT in an in-line irradiation coil. Optimal condition for maximum yield of photolysis; pH<1. D.L. 2 ng. Injection 100 μl.	TBT analysis in seawater Extraction with chloroform, evaporation and redissolving in 10 ml methanol	181
As	Flame AAS using on-line hydride generation Hydrogen diffusion flame was burnt on an inverted Y glass burner	Hypersil ODS, 250 × 5 mm i.d. Eluent: sulphuric acid 1.8 × 10 ⁻⁵ M at 0.5 ml min ⁻¹ for 5 min, then 1.5 ml min ⁻¹	FAAS linear range and DL are compared to FAFS and ICP-AES Linear range 0.2–6.0 μg DL 10–43 ng Injection 1 ml	As ^{III} , As ^V , MMA and DMA in pore water	176
Pb	Flame AAS using continuous ethyl derivatization Vapour swept to the quartz cell	Guard column (Nucleosil C ₁₈), 300 × 4.6 mm i.d. Eluent: methanol/water (2+8) and 600 μg ml ⁻¹ tetramethylenedithiocarbamate at 1 ml min ⁻¹	Linear up to 5 ng for alkyllead DL ionic alkyllead compounds 0.1 ng, inorganic lead 10 ng	Synthetic mixture of ionic methyl- and ethyl-lead compounds	175

Hg	Flame AAS using continuous mercury vapour generation by heat Vapour swept to the quartz cell	Zorbax ODS column, 250 × 4.6 mm i.d. Eluent: methanol/0.05 M ammonium acetate at pH 5.7 (3 + 2), containing 0.01% 2-mercaptoethanol at 0.7 ml min ⁻¹	Linearity up to 500 ng DL (2σ) 0.6 ng Total analysis 45min Injection 100 μl	Methyl-, ethyl- and phenyl-mercury in standards and methyl-mercury in fish	183
As	Flame AAS using thermospray nebulization and thermochemical hydride generation Cool diffusion H ₂ /O ₂ flame atomizer	Cyanopropyl bonded phase column, 150 × 4.6 mm i.d. Eluent: methanoic mobile phase containing 30% diethylether, 0.05% triethylamine and 1% acetic acid at 0.2–0.5 ml min ⁻¹	Linear from 50 to 1000 ng as the iodide salts DL 8–14 ng as free cation Injection 100 μl	As ^V , DMA, arsenocholine, arsenobetaine, and tetramethylarsonium cations	182
Hg	Flame AAS using continuous mercury vapour generation by means of tin(II) chloride oxidation	Develosil-ODS (30 μm) precolumn, 27 × 0.51 mm i.d. and STR-ODS-H (5 μm) μ-column, 125 × 0.50 mm i.d. Eluent: 0.04 M cysteine in 0.1 M acetic acid at 200 μl min ⁻¹	Linear up to 5 ng mercury DL (3σ) 0.1 ng Injection 100 μl	Mercury(II) chloride, Methylmercury chloride and ethylmercury chloride in waste water	184
As	Flame AAS using continuous hydride generation Quartz absorption cell flame heated	Nucleosil 10SB column, 250 × 4.4 mm i.d. Eluent: 0.05 M phosphate buffer (pH 6.775) at 0.5 ml min ⁻¹	Optimisation of the hydride generation is given DL 2.6 ng at 50 μl injection Injection 50 μl	As ^{III} , As ^V , MMA and DMA in liver supernatant and in seaweed extract	169
Applications using HPLC-plasma detectors					
Pb	ICP with direct coupled concentric nebulizer	LiChrosorb RP-2 (10 μm), 120 × 4.6 mm i.d. Eluent: butanol/ethanol/water (15:35:50) at 1 ml min ⁻¹	Linear up to 500 ng DL (2σ) TML 2 ng and TEL 11 ng 50 μl injection	Tetraalkyllead compounds in gasoline	206

TABLE 16.3 (continued)

Element	Detector	Chromatography	Comments	Sample	Ref.
As	ICP with conventional cross-flow nebulizer using continuous hydride generation	Excalibar Spherisorb ODS (5 μm), 150 \times 4.6 mm i.d. Eluent: 0.005 M tetrabutylammonium phosphate in water at 1 ml min ⁻¹	Linear up to 500 ng DL (3 σ) 10 ng for inorganic As and 20 ng for DMA First HPLC-ICP system using continuous hydride generation Comparison with directly coupled HPLC-ICP 200 μl injection	As ^{III} , As ^V and DMA in well water	197
Pb	ICP using a glass-frit nebulizer for RP-HPLC and a concentric nebulizer for IE-HPLC	Different RP columns for tetraalkyllead, e.g. Spherisorb C ₁₈ -ODS (10 μm), 120 \times 4.6 mm i.d. Eluent: isopropanol/water (60:40) at 1 ml min ⁻¹ Ion-exchange column for trialkyllead; Partisil-10 SCX, 250 \times 4.6 mm i.d. Eluent: 0.03 M ammonium acetate in methanol/water (70:30) at 1 ml min ⁻¹	TML and TEL linear up to 300 ng DL (2 σ) for TML and TEL are 67 and 96 ng resp. with a glass-frit nebulizer (high DL caused by foaming in the nebulizer) Concentric nebulizer; DL TML 2 ng and TEL 11 ng Clogging of the glass-frit nebulizer when millimolar levels of salt solutions are used 50 μl injection	Synthetic solutions of tri- and tetraalkyllead compounds	196

As	ICP	Hamilton PRP-1 column (10 μ m), 250 \times 4 mm i.d. Eluent: 0.05 M sodium heptanesulfonate in 2.5% aqueous acetic acid at 2 ml min ⁻¹	DL MMA 3 ng, DMA 5 ng and AsChol 14 ng Extensive sample clean-up 100 μ l injection	As ^{III} , As ^V , MMA, DMA, AsBet and AsChol in crabmeat	201
Sn	ICP with thermospray nebulizer	Reversed-phase ionpair chromatography, no details	Excellent resolution of the species, and a tenfold improvement in the element-selective detection reported, no details	Dibutyltin, tributyltin and triphenyltin	204
As	ICP, HPLC directly coupled into the standard cross-flow nebulizer	Aminex A-27 Radial-PAK, 100 \times 8 mm i.d. column Eluent: linear elution eluent from water to 0.5 M (NH ₄) ₂ CO ₃ in 15 min, followed by an isocratic flow of 0.5 M (NH ₄) ₂ CO ₃ at 1 ml min ⁻¹	Linear up to 8 μ g DL (2 σ) As ^{III} 390 ng, As ^V 126 ng, MMA 57 ng and DMA 60 ng Injection 10–500 ml	As ^{III} , As ^V , MMA and DMA in culture cell medium	199
As	ICP with DIN 15% of the column effluent into the capillary tube of the nebulizer	Partisil 5 ODS-3 column, 250 \times 4.6 mm i.d. Eluent: 0.005 M tetrabutylammoniumphosphate in water/methanol (9:1) at 0.75 ml min ⁻¹	DL (3 σ) 12–20 ng Injection 200 μ l	Synthetic mixture of As ^{III} , As ^V , MMA and DMA	200
As	ICP using continuous hydride generation	Hypersil ODS (3 μ m), 250 \times 5 mm i.d. Eluent: 1.8 \times 10 ⁻⁵ M sulphuric acid at 0.5 ml min ⁻¹ for 5 min, then 1.5 ml min ⁻¹	Linear up to 20 μ g ml ⁻¹ DL (2 σ) As ^{III} 51, As ^V 128, MMA 140 and DMA 112 ng ml ⁻¹ Injection 1000 μ l	Synthetic mixture of As ^{III} , As ^V , MMA and DMA	176

TABLE 16.3 (continued)

Element	Detector	Chromatography	Comments	Sample	Ref.
As	ICP with a thermospray nebulizer interface	Dionex HPIC AS4A column Eluent from 100% deionized water to 0.05 M ammoniumcarbonate containing 0.2% methanol at 1 ml min ⁻¹	Thermospray nebulizer is compared with pneumatic nebulizer DL (3σ) without HPLC but as flow injection in the ion chromatographic mobile phase as carrier stream; As ^{III} 234, As ^V 3.4, DMA 31 and PhAs 2.4 ng Injection 200 μl	Synthetic mixture of As ^{III} , As ^V , DMA and PhAs	201
As	ICP using continuous hydride generation G-L separator outlet directly coupled to the plasma	Nucleosil SB column (5 μm), 200 × 4.6 mm i.d. Eluent: phosphate buffer (0.05 M) pH 6.75 at 1 ml min ⁻¹	DL (2σ) As ^{III} 0.35, As ^V 0.92, MMA 0.38 and DMA 2.13 ng Injection 100 μl	Synthetic mixture of As ^{III} , As ^V , MMA and DMA	202
As	Helium alternating current plasma (ACP) using continuous hydride generation G-L separator directly coupled to the plasma	Spherisorb ODS (5 μm) column, 150 × 4.6 mm i.d. Eluent: 0.005 M tetrabutylammonium phosphate in methanol/ water (3:97) at 1 ml min ⁻¹	DL (3σ) As ^{III} 2.8, As ^V 4.0 and DMA 3.0 ng Injection 200 μl	River and tap water spiked with As ^{III} , As ^V and DMA	203

Sn	ICP with a Hildebrand grid nebulizer	Spherisorb ODS-2 (5 μ m), 250 \times 4.6 mm i.d. I.P. column Eluent: 0.004 M sodium pentane sulphonate in methanol/water/acetic acid (80:19:1) acidified with 1 M sulphuric acid to final pH 3.00 at 1 ml min ⁻¹ Adsobosphere SCX (5 μ m) I.E. column, 250 \times 4.6 mm i.d. Eluent: 0.1 M ammoniumacetate in methanol/water (85:15) at 1 ml min ⁻¹	DL (3 σ) for IP chromatography (for IE); TMT 200 (450), TBT 1700 (800) and TphT 1700 (1500) ng HPLC-ICP used to determine optimum conditions for HPLC-ICP/MS Injection 200 μ l	Synthetic solution of trimethyl-, tributyl- and triphenyl-tin	205
As	ICP with an ultrasonic nebulizer	Partisil 5 ODS-3 (10 μ m), 250 \times 4.6 mm i.d. Eluent: 0.05 M tetrabutylammonium phosphate in water at 1.0 ml min ⁻¹	DL 6–9 ng Improved nebulization efficiency (10–30%) compared to pneumatic nebulization (1–3%) Injection 200 μ l	As ^{III} , As ^V , MMA and DMA in groundwater	207
Applications using HPLC-ICP-MS					
Hg	ICP-MS using post-column mercury cold-vapour generation Spray chamber (glass) cooled to 8°C	PicoTag C- ₁₈ Eluent: 0.06 M ammonium acetate, 3% acetonitrile and 0.005% 2- mercaptoethanol at a flow rate of 1.0 ml min ⁻¹	Linearity (DL 2 σ): Hg up to 13 ng (120 pg), MeHg up to 60 ng (60 pg), (Et)Hg up to 120 ng (120 pg) and thimerosal (without cold- vapour generation) up to 4000 ng (2 ng) Results compared to analysis without cold-vapour generation Injection 100 μ l	Methylmercury in tuna fish, thimerosal in contact lens solutions, monitoring the ²⁰² Hg isotope	210

TABLE 16.3 (continued)

Element	Detector	Chromatography	Comments	Sample	Ref.
Sn	ICP-MS using a Hildebrand grid nebulizer Cooled spray chamber	For IP chromatography; Spherisorb ODS-2 (2 μm), 250 \times 4.6 mm i.d. Eluent: 0.004 M sodiumpentane- sulphonate in methanol/water/acetic acid (80:19:1) at pH 3.0 at 1 ml min^{-1} For IE chromatography: Adsobosphere SCX, 250 \times 4.6 mm i.d. Eluent: 0.1 M ammonium acetate in methanol/water (85:15) at 1 ml min^{-1}	Linear range for IP = 2 and IE = 3 orders of magnitude DL (3 σ) for both IP and IE chromatography; TMT = 0.4 ng (both), TBT 1 ng (both) and TPhT 0.7 and 0.8 resp. Injection 200 μl	Synthetic solutions of triorganotins; TMT, TBT and TPhT, monitoring the ^{120}Sn isotope	205
Sn	ICP-MS with a concentric nebulizer and a cooled spray chamber	Micellar LC using Spherisorb ODS-2 (5 μm), 250 \times 4.6 mm i.d. column Gradient elution from 0.02 M to 0.1 M sodiumdodecylsulphate (SDS) in 2 min. and constant 0.1 M SDS after 2 min	DL (3 σ) for TMT, TET and TPrT are 27, 51 and 111 pg resp. at 0.1 M SDS. MMT, DMT and TMT; 46, 26 and 126 pg resp. at 0.02 M SDS Linear range; 3.5 orders of magnitude Injection 100 μl	Synthetic solutions of organotin compounds; MMT, DMT, TMT, TET, TPrT and TBT and TPhT, monitoring the ^{120}Sn isotope	211

As	ICP-MS with a Hildebrand grid nebulizer and a cooled spray chamber	Adsorbosphere-NH ₂ (5 μ m), 250 \times 4.6 mm i.d. Eluent: 0.020 M NH ₄ H ₂ PO ₄ and 0.002 M CH ₃ COONH ₄ in methanol/water (30:70) at 1 ml min ⁻¹	Linear range; over 2 orders of magnitude DL in synthetic solutions (urine); As ^{III} 38 (73) pg, As ^V 91 (96) pg, MMA 44 (36) pg and DMA 20 (38) pg Injection 200 μ l DL (3 σ) 50–300 pg Anion pairing generally more sensitive to changes in the matrix Anion exchange more tolerant through higher buffer capacity of the mobile phase Cation pairing used for DMA and AsBet in biological samples	As ^{III} , As ^V , MMA and DMA in urine	214
As	ICP-MS	IP and IE chromatography using seven different columns and different mobile phases	Injection 50 or 200 μ l DL (3 σ) As species 0.6 pg except for MMA; 0.5 pg MMT and DET 10 pg, DMT 8 pg and TMT 9 pg Arsenic species linear up to 10 ng Injection 0.5 μ l	As ^{III} , As ^V , MMA, DMA and AsBet in synthetic solutions and marine biological reference material DORM-1	215
As	ICP-MS using a direct injection nebulizer (DIN)	Inertsil ODS-2, 100 \times 1 mm i.d. Eluent: 0.005 M heptyltriethylammonium phosphate in methanol/water (5:95), pH 6.0 for separation of As species and 0.005 M ammonium heptanesulfonate in methanol/water (25:75), pH 3.1 for tin species both at 30 μ l min ⁻¹		Arsenic species; As ^{III} , As ^V , MMA and DMA Tin species; MMT, DMT, DET and TMT; both in synthetic solutions	212

Methyl- and ethyltins can also be analyzed with the system presented in Fig. 16.9, after mineralization of the alkyltin compounds [168]. For methylated tin compounds, this technique was found to be superior to the H/CT/GC/QFAAS determination procedure since, in their experiments, redistribution reactions did not take place on the chromatographic column. Detection limits were of the same order of magnitude as for H/CT/GC/QFAAS; e.g. 8–14 pg as Sn. For tetramethyl- and tetraethyltin, the response was similar to that of inorganic tin; for other compounds, the response was a function of the thermal stability and volatility of the alkyltin hydrides produced.

To overcome the problem of detecting only volatile hydride species, Ebdon and co-workers modified the arrangement displayed Fig. 16.10. They introduced an “on-line” UV photolysis coil between the HPLC and the hydride generator [164,165,176,180]. This approach allows the determination of non-volatile species by hydride generation. A detection limit of 2 ng TBT can be obtained in this combined postcolumn UV-photolysis hydride-generation system.

Recent developments in the HPLC-furnace interface include a thermospray micro-atomizer operated at 1000°C. The HPLC effluent is flash evaporated to an aerosol before introduction into the furnace [182]. The interface is based on thermospray nebulization of the HPLC methanolic eluent, pyrolysis of the analyte in a methanol/oxygen flame, gas-phase thermochemical hydride generation using excess hydrogen, and cool diffusion flame atomization of the resulting arsine in a quartz cell mounted in the AAS optical beam. The thermospray is effective for effluents containing up to 50% of water. This on-line interface has been used successfully for the speciation of organoarsenic compounds, including arsenobetaine, arsenocholine and tetramethylarsonium salts with detection limits of 13.3, 14.5 and 7.6 ng, respectively.

For non-hydride forming elements such as mercury, a similar interface to that given in Fig. 16.10 was developed. The eluting mercury compounds are converted into Hg^0 vapour by heating [183] or by chemical reduction with tin(II) chloride [184], and sweeping of the generated vapour through a quartz-windowed cell aligned in the light path of an AAS. A detection limit of 0.1 ng mercury could be obtained [184] by chemical reduction with tin(II)chloride whereas the heat conversion technique yielded a detection limit of 0.6 ng [183]. A summary of several applications is given in Table 16.3.

16.5.1.3. Peak storage technique in HPLC-FAAS

Finally, another hyphenation between an HPLC and a FAAS transports the sample to the detector by means of rotating spirals [165]. The HPLC eluates are collected as a series of discrete amounts and transported by a series of rotating spirals to the flame of an AAS. Once the samples are loaded on the spirals, the mobile phase is evaporated in a thermal zone prior to entering the flame where atomization and detection will occur. This interface has been successfully used for the direct determination of di- and tri-alkyllead compounds.

15.5.2. Electrochemical atomization (HPLC-GFAAS)

The graphite furnace (GFAAS) offers the advantage of high sensitivities for small amounts of sample but the sequential nature of drying and ashing steps prior to atomiza-

tion make it difficult to couple the continuous flow of the HPLC effluent. In a first approach, an autosampler device is used to sample the HPLC effluent in a PTFE flow-through cell and to inject an aliquot (typically 10–50 μl) into the furnace [185,186], generating pulsed signals for the analyte (Fig. 16.11). In a second type of approach, the effluent can also be stored as discrete fractions in the fraction collector. After full elution of the fractions, they can be injected off-line into the GFAAS [185,187]. This later approach was used by Vickrey et al. [187,188]. They stored the eluate containing the analytes during the chromatographic run. The capillary tube containing the analytes was then analyzed automatically off-line by GFAAS (Fig. 16.12). This last method yields more data points

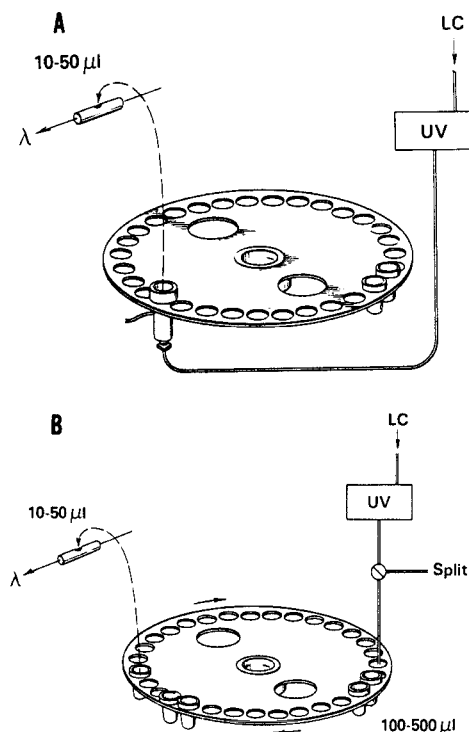


Fig. 16.11. Two views of the carousel GF sample holder of the AS-1 autosampler are depicted: (A) in the pulsed (periodic stream sampling) mode, the AS-1 sampling pipette traverses the arc (-----) between the GF tube orifice (at arrowhead) and the conical aperture to the well sampler. Thus, 10–50 μl effluent samples are reproducibly and periodically introduced for automatic, programmed GFAAS analysis at the chosen wavelength. Here, effluent from the HPLC assembly continuously passes through the well sampler with the AS-1 carousel in a fixed position. (B) In the survey (segmental stream analysis) mode, the AS-1 carousel is permitted to revolve normally at a rate mainly dependent on the column flow, extent of effluent stream splitting, needed fraction size (or chromatogram resolution), analyte concentration and GFAA sample size chosen. The period between presentation of a new cup to the effluent is set by the combination of the GF atomization timing program and the number of replicate GF samples pipetted from each cup. Conventional 1 ml manufacturer's polycarbonate carousel cups are employed if these are compatible with the mobile phase liquids (reproduced from [185] with permission from Preston Publications).

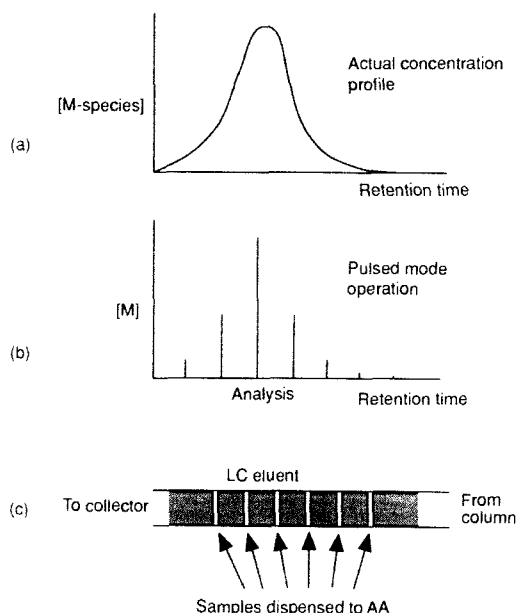


Fig. 16.12. Description of the "pulsed" sampling mode. (A) actual concentration profile of the metal species leaving the column. (B) The measured concentration profile from the intermittent removal of aliquots of the eluent stream. (C) A diagram of the eluent stream showing the aliquots which are removed and analysed by the graphite-furnace atomic absorption spectrometer (reproduced from [222] with permission from Taylor & Francis).

per chromatographic peak, resulting in a better signal to noise ratio. Both methods were demonstrated to be suitable for the speciation of Sn [185,188], Hg [185], As [185,189] and Pb [185–188] compounds. The detection limits quoted were in the range of 0.5–100 ng as organometal [187,189]. All of these techniques generate pulsed signals, whose sum is used for quantitation of the chromatographic peak. These systems do not provide a continuous, on-line, real-time analysis. The accuracy of the determination mode depends on the number of pulses detected.

Recent improvements in HPLC-GFAAS interfacing have been proposed by Nygren et al. [190]. They used a fused silica capillary heated through stainless steel tubing (200°C) where the effluent is volatilized to aerosols. These aerosols are directly introduced into a vitrified graphite tube. Limitations of this approach are caused by the solvents and buffer solutions in the eluent generating soot and degrading the baseline. This technique was applied to determinations of di- and tributyltin species. Calibration curves are linear in the 1–100 ng range with detection limits of 0.5 ng.

However, despite the better overall sensitivity of the GFAAS, coupling with HPLC is troublesome. Interfacing is difficult, mainly owing to the discontinuous nature of the GFAAS. Furthermore, GFAAS is poorly suited to the large volumes of solvents that are normally employed in HPLC.

16.5.3. High performance liquid chromatography interfaced to plasma emission detectors (HPLC-PED)

In comparison to hyphenation between HPLC and GFAAS or FAAS, the plasma source has the advantages of multi-element operation, easy coupling to the chromatography, responsiveness for metals and non-metals and the acceptance of the continuous flow of the HPLC eluent. Important disadvantages are associated with the sensitivity of the plasma to organic solvents and the general overall inefficiency of nebulizer systems. Three principal plasma sources which have been evaluated as liquid chromatography detectors are microwave-induced plasma (MIP), direct current plasma (DCP) and inductively coupled plasma (ICP)

The hyphenation potential of low power helium MIP is limited since the continuous HPLC flow will quench the discharge. New capillary columns with $\mu\text{l min}^{-1}$ flows can be a solution to these problems in MIP interfacing, but then the sample capacity may limit any application to trace analysis.

DCP appears to offer certain advantages with regard to HPLC coupling. It appears to give a more stable plasma, especially with the introduction of mixed organic/aqueous eluents. The plasma stability will be superior to that obtained with ICP plasmas [191]. However, with DCP direct interfacing, there is the problem of high detection limits, in the $100 \mu\text{g l}^{-1}$ range, so the environmental applicability will be limited [191]. The coupling of HPLC and DCP has been described for arsenic speciation [192]. The sensitivity was poor, being only 20% of that obtained with an ICP.

Inductively coupled plasma (ICP) discharge was first developed in the 1960s and is now the most widely used spectrochemical source [193]. The major difficulty experienced in HPLC-plasma hyphenation is the incompatibility of the system with mobile phases at typical analytical flow rates. Poor detection limits are generally reported for HPLC-ICP because of the inefficient conversion of effluents into aerosols and their transport to the plasma. Typically, only 1–5% of the sample reaches the plasma torch. A frequent observation has been that there is poor tolerance of the ICP for the mobile phases commonly used in HPLC, particularly with ion-pairing or size-exclusion LC separation techniques. Techniques to overcome these problems are directly related to improving nebulization rates and the transmission of the analyte to the plasma.

Different types of nebulizers are used in interfacing HPLC to ICP. Some general characteristics are as follows [194,195]:

- The concentric nebulizer is very satisfactory for many sample solutions but tends to clog, especially with low aspiration gas flow rates. Furthermore, poor transport efficiency occurs with low gas flow rates.
- The cross-flow nebulizer is similar to concentric nebulizer.
- The glass-frit nebulizer is capable of handling the organic solvents commonly used in reversed phase HPLC. Solvents with a high surface tension generate important foaming which severely affects the efficiency of the nebulizer [196].
- The thermospray nebulizer yields 100% efficiency in aerosol production at flow rates of about 1 ml min^{-1} or more and offers optimal conditions for HPLC-ICP interfacing [195].

- The dual platinum grid (Hildebrand) nebulizer is capable of handling samples with a high salt content.

Most of the HPLC-ICP systems have been applied to the speciation of arsenic [176,197–203], but organotin [204,205] and organolead compounds [196,206] have also received some attention.

The concentric- and the cross-flow nebulizer are commonly used in hyphenated HPLC-ICP systems. They are now being replaced by the direct introduction nebulizer (DIN), or the thermospray and ultrasonic nebulizers which offer higher efficiency of sample-introduction. Determinations of alkyllead compounds have been performed with HPLC-ICP fitted either with a concentric [206] or a glass-frit nebulizer [196]. The concentric nebulizer gave a 10–30-fold improvement in the detection limits for TML and TEL. The difference is mainly explained by the foaming and clogging problems associated with the use of the glass-frit nebulizer.

Similarly, HPLC-ICP has been applied to organotin determinations [204,205]. However, the detection limits are poor ranging from 200 to 1770 ng and thus limiting environmental applications of this system. As with FAAS, determinations of arsenic species have also been performed, with and without continuous postcolumn hydride generation using different types of nebulizers (Table 16.4).

The nebulizers are critical for environmental applications since they directly control the overall analyte transfer to the plasma. HPLC has been interfaced directly to conventional pneumatic nebulizers of an ICP, yielding detection limits in the nanogram range for arsenic compounds [197,199,201]. Overall sensitivity improvements can be obtained by using the DIN interface [200]. The use of thermospray sample introduction to facilitate coupling between the HPLC and ICP offers substantial improvements, with reduced detection limits for most As species [201]. Up to now, the best results for interfacing HPLC

TABLE 16.4

DETECTION LIMITS OBTAINED FOR HPLC-PED SYSTEMS FOR ARSENIC SPECIATION

	As ^{III}	As ^V	MMA	DMA	PhAs	Ref.
HPLC-ICP	960	480	4000			197
conventional	390	126	57	60		199
nebulizer	50 ^a	60 ^a		50 ^a	50 ^a	201
HPLC-DIN-ICP	19.6		11.2			200
HPLC-USN-ICP	6	9	3 ^b	3 ^b		207
HPLC-TS-ICP	234	3.4		31	2.4	201
HPLC-HG-ACP	3	4		3		203
HPLC-HG-ICP	10	10		21		197
	51	128	140	112		175
	0.3	0.9	0.4	2.1		202

^aDetection limit given for flow injection analysis using the mobile phase of the chromatographic system.

^bEstimated.

with ICP have been achieved using an ultrasonic nebulizer as the sample introduction device [207]. An ultrasonic nebulizer gives an average 10-fold improvement in nebulization efficiency (i.e. 10–30%) compared to a pneumatic nebulizer (1–3%). Further, the desolvation which occurs in the nebulizer system removes most of the solvent (water and organic solvent) resulting in improved plasma torch conditions and better overall signal-to-noise ratio.

As with FAAS, the on-line continuous hydride generation after HPLC separation can be used in interfacing to a plasma emission detector (PED) [176,197,202,203]. The use of a gas-liquid (G-L) separator avoids entry of the mobile phase and the hydride generation reagents into the nebulizer, resulting in very low background signal. Detection limits in the low nanogram range have been reported by Colon and Barry [203]. They used an alternating current plasma emission source (ACP) after postcolumn hydride generation. The best detection limits with postcolumn hydride generation have been reported by Rauret et al. [202]. They achieved limits for four As compounds in the sub-nanogram range, 0.3–2 ng. This makes the method suitable for most environmental applications. A summary of several applications using HPLC-PED is given in Table 16.3.

16.5.4. High performance liquid chromatography interfaced to inductively coupled plasma/mass spectrometry (HPLC-ICP/MS)

One of the limiting factors for hyphenated techniques using HPLC-PED is definitely the lack of overall sensitivity due to the sample introduction mode. The high sensitivity of the ICP/MS detector compensates for this sensitivity reduction and then offers sub-ng limits of detection, comparable to those for ICP/AES detectors. When ICP/MS is coupled to HPLC, a very sensitive and element-selective analytical system for speciation analysis is obtained. The twofold identification system produces the most reliable and specific identification of the analyte for environmental applications. Detection limits in the low picogram range are within reach, and are 2–3 orders of magnitude lower than those obtained with FAAS and ICP detectors.

The ICP/MS detection system is widely used because of its good sensitivity (1–20 pg ml⁻¹), the provision of generally uncomplicated mass spectra, and its capability for isotope ratio determination [208]. The high sensitivity of the system also eliminates the need for a postcolumn derivatization step for most applications [209]. Interfacing between the HPLC column and the ICP/MS detector is quite straightforward (Fig. 16.13).

One of the first interfaces realized was used to determine methylmercury in fish tissues [210]. A postcolumn mercury cold-vapour generation was used prior to entrance into the ICP/MS, yielding a detection limit ten times better than direct HPLC-ICP/MS analysis with 6% RSD. Organotin determinations have also been performed by HPLC-ICP/MS [205,211,212], using ion pairing (IP), ion exchange (IE) and micellar liquid chromatography (MLC) for separation of the organotin compounds. The MLC separation mode gave the best results in terms of detection limits (27 pg for TMT in comparison to 400 pg for IP and IE liquid chromatography). However, good separation of alkyltin compounds was only obtained for small alkyl chains. Butyltin compounds cannot be detected by this HPLC-ICP/MS approach owing to their higher *k'* values associated with the molarity of the mobile phase. Increasing the molarity of the mobile phase led to clogging of the

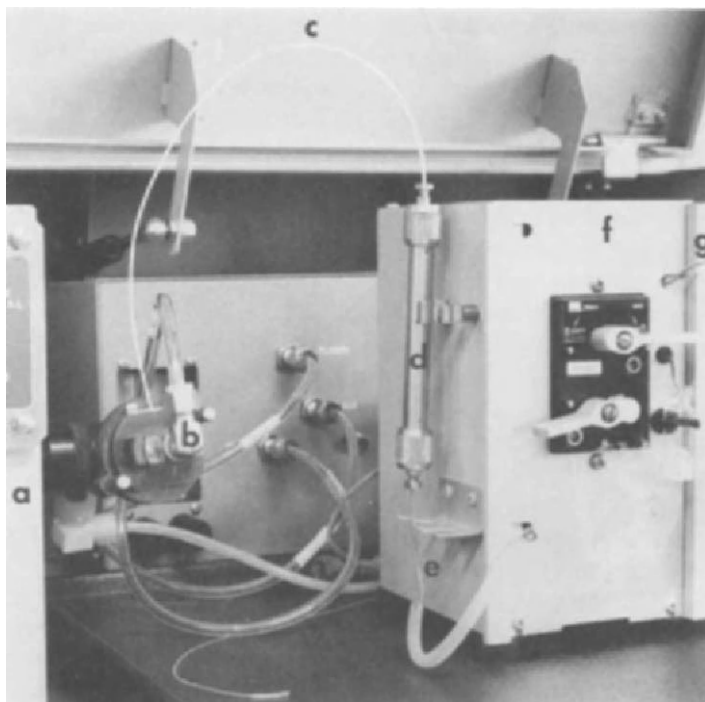


Fig. 16.13. Hyphenation between HPLC and ICP/MS for the speciation of arsenic. (a) Torch box of the ICP/MS (PE Elan 5000); (b) standard cross flow nebulizer; (c) PTFE tubing (0.5 mm i.d.); (d) HPLC column (Chrompack Ionospher-C cation exchange column); (e) liquid out; (f) injector valve (Waters); (g) liquid in (courtesy of E.H. Larsen, Levnedsmiddelstyrelsen, National Food Agency of Denmark/Ministry of Health, Soborg, Denmark).

sampling orifice of the ICP/MS. Houk and co-workers described an other system interfacing a micro-bore LC and an ICP/MS, using a direct injection nebulizer (DIN) [212]. This interface injects all of the sample into the ICP and has a dead volume of less than $1\ \mu\text{l}$. Absolute detection limits obtained are in the range of 8–10 pg. They represent a significant improvement over those obtained previously by HPLC-ICP/MS [205,208,211], but are inferior by an average factor of 200 to the Sn detection limit values obtained by the hyphenation of supercritical fluid chromatography (SFC) with ICP/MS detection [213]. Relative detection limits are however poor due to the low injection volume of $0.5\ \mu\text{l}$ of the analytes.

Arsenic speciation has also been significantly addressed by the use of HPLC-ICP/MS [212,214,215]. Detection limits for four arsenic species, As^{III} , As^{V} , MMA and DMA in samples such as urine and biological reference materials are in the range of 40–100 pg and 50–300 pg, respectively. The micro-bore HPLC-ICP/MS system with the DIN interface has also been used for As speciation [212]. Again, as has been observed for organotin speciation, the absolute detection limits of 0.6 pg are superior by a factor of

50–500 to those obtained previously by HPLC-ICP/MS using conventional nebulizers [214,215]. However, the relative detection limits are poor owing to the low injection volume of $0.5\ \mu\text{l}$ of the analytes.

Although supercritical fluid chromatography (SFC) does not strictly fall within this category, it can be interfaced successfully to atomic spectrometric detectors (Fig. 16.14). Capillary SFC is gaining popularity, especially for the separation of high molecular weight compounds, and bridges the gap between GC and LC in many respects. Although it combines the properties of GC and LC, SFC does not suffer from some of the problems inherent in the LC separation approach since the mobile phase is a gas at atmospheric pressure [159]. The column effluent is therefore compatible with many GC detectors. SFC has also been successfully coupled to an ICP/MS detector [213]. It displays high potential for the determination of environmentally significant levels of organometallic species. A good separation of tetraalkyltins was performed with supercritical CO_2 using a capillary column, $2.5\ \text{m}$ long \times $50\ \mu\text{m}$ i.d., coated with a $0.25\ \mu\text{m}$ film thickness of SB-Octyl-50, using supercritical CO_2 . Detection limits for tetrabutyltin and tetraphenyltin were 0.034 and 0.047 pg, respectively, at an injection volume of 10 nl. Linear working ranges are given over three decades (1–1000 pg). General applications using HPLC-ICP/MS are given in Table 16.3.

15.5.6. High performance liquid chromatography coupled to other detectors

Speciation of organometallics can finally be achieved by combining HPLC with several other detectors such as ultraviolet (UV) absorption spectrometry, atomic fluorescence spectrometry (AFS), electrochemistry, laser-enhanced ionization (LEI) and laser excited

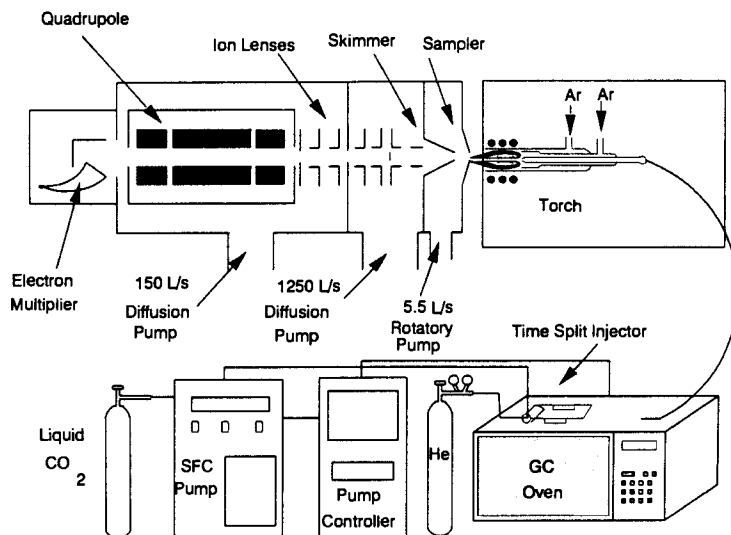


Fig. 16.14. SFC-ICP/MS system diagram and SFC-ICP/MS interface (reproduced from [213] with permission © American Chemical Society).

atomic fluorescence spectrometry (LEAFS) [216–221]. The highest sensitivity suitable for environmental application are obtained with the last three techniques mentioned.

HPLC separation coupled to reductive amperometric electrochemical detection resulted in good sensitivities for nine organomercury compounds. Detection limits of 100–200 pg have been reported by Evans and McKee [217], with a preliminary system that was not fully optimized. The apparatus uses a reversed phase LiChrosorb RP-18 column, a BAS Model LC-4B/17 amperometric controller and transducer for HPLC, a BAS TL-6A electrochemical cell (using a gold working electrode) and an Ag–AgCl (3 M NaCl) reference electrode.

Significantly higher sensitivity can be obtained by using the LEI detection mode. In this technique, two pulsed lasers are used to produce a double resonance electronic excitation state of the tin atoms present in a flame. These atoms undergo rapid collision and ionization and are quantified by the electrodes in the flame. The high sensitivity of the LEI detection mode originates from its ability to ionize virtually every atom irradiated by the laser and from the high detection efficiency. The main drawback of LEI is the occurrence of potential interferences generated from easily ionized elements. Epler et al. [219] applied this technique to the determination of alkyltins. Separation was achieved by a Partisil-10 SCX cation exchange column with a 75:25 methanol/water eluent, 0.05 M in ammonium acetate buffer (pH 5.1), at 2 ml min⁻¹. The LC column was connected to the pneumatic nebulizer of the LEI through a 30-cm piece of polyethylene tubing (0.3 mm i.d.). The absolute detection limit for TBT is 60 pg and is equivalent to HPLC-ICP/MS performances (26–126 pg) [211].

Finally, hyphenation between HPLC and flame-laser-excited atomic fluorescence spectrometry (LEAFS) also allows excellent detection limits for organomanganese and trialkyltin compounds, at 10–22 pg and 240 pg, respectively.

16.6. CONCLUSION

The wide array of hyphenated techniques described in this chapter illustrates the important technical progress made during the last decade. However, not one technique can substitute for all the others and they should in general be considered as complementary. If instrumental solutions can be considered satisfactory, very few are available commercially at present. The success of speciation information delivered is not only dependent on the instrumentation used. The growing demand for speciation analysis is still hampered by the cost of the solutions available and the sample through-put. In this respect, systems using on-line cryofocusing and chromatographic separation prior atomic spectrometry detection appear to be robust and sensitive, allowing a high sample through-put. Despite these remarks, the high cost represented by the hyphenation of HPLC to ICP/MS will certainly expand considerably our understanding of speciation and its importance in biological processes.

However, the determination of chemical species at ultra-trace levels in environmental samples has renewed the need for a strong analytical chain prior to instrumental determination. Contamination-free sampling has become most important since levels detected are extremely low. Stability during sample storage may be one of the most critical steps at the

present time for environmental speciation analysis. Redox species are particularly unstable. Methylation, degradation or loss via volatilization of some organometallic species may alter the correct determination of organometallic compounds initially present in the sample. Finally, sample pretreatment can significantly modify the yield of species detected in the sample. The sample pretreatment stage now represents the new challenge for significant progress in speciation analysis.

APPENDIX: LIST OF ABBREVIATIONS

AAS, atomic absorption spectrometry; AED, atomic emission detection; AES, atomic emission spectrometry; AFS, atomic fluorescence spectrometry; CT, cryogenic trapping; D, derivatization; DCP, direct current plasma; DIN, direct injection nebulizer; DMA, dimethylarsinate; ECD, electron capture detection; F, flame; FIA, flow injection analysis; FID, flame ionization detector; FPD, flame photometric detection; GC, gas chromatography; GF, graphite furnace; HG, hydride generation; HPLC, high performance liquid chromatography; ICP, inductively coupled plasma; IE, ion exchange; IP, ion pairing; LC, liquid chromatography; LEI, laser enhanced ionization; LEAFS, laser excited atomic fluorescence spectrometry; MLC, micellar liquid chromatography; MMA, monomethylarsonate; MS, mass spectrometry; QF, quartz furnace; PED, plasma emission detection; SFC, super critical fluid chromatography; TBT, tributyltin; TEL, tetraethyllead; TML, tetramethyllead; UV, ultraviolet;

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Chapter 17

The potential of capillary electrophoresis in environmental analysis

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17.1. GENERAL INTRODUCTION

17.1.1. Capillary electrophoresis and its position in modern analytical separation science

Electrophoresis and its theory date back to the end of the last century, so one might wonder why it took so long to become the trend in analytical separation science that it is nowadays. Some practical breakthroughs in recent years were prerequisites, and should be mentioned here. Hjertén [1] described the use of narrow-bore glass tubes for zone electrophoresis for the first time in 1967. The inner diameters, however, were still about

3 mm. Mikkers et al. [2] described the use of sub-mm PTFE capillaries (typically 200 μm i.d.) for the separation of both inorganic and organic ions, in 1979. Many authors refer to the important experimental breakthrough described by Jorgenson and Lukacs [3] in 1981. Their zone electrophoretic separations were carried out in 75–100 μm i.d. glass capillaries at a voltage of 30 kV. Due to the high field strength and the efficient heat dissipation provided by the capillary format, the separation efficiencies were very high, and are nowadays typically between 100 000 and 600 000 theoretical plates. The introduction of a sophisticated commercial instrument in 1989 [4] was followed by several instruments from other companies in the last few years. A comprehensive reference and textbook, covering all aspects of capillary electrophoresis has been published recently [5]. Relevant information can also be found in other books [6–8] and in two recent reviews [9,10].

One might question the introduction of another high performance analytical separation method. Does it bring applications which cannot be carried out simply by well-established techniques such as capillary gas chromatography (GC) and high performance liquid chromatography (LC)? The most practiced mode, capillary zone electrophoresis (CZE), is used for the separation of ionic and ionizable compounds having sufficient water solubility. These types of analytes are normally not very amenable to GC; at least derivatization will be required. The major competition comes from the LC field (ion-exchange, ion-exclusion and ion-pair chromatography, and reversed-phase chromatography). In general, CZE will show much higher plate numbers, shorter analysis times and a different selectivity compared to LC systems. The different selectivity makes CZE complementary to LC. Single peaks in LC might consist of two or more compounds, as shown by a CZE experiment. In this sense, unique separations can be expected. When comparing similar applications of LC and CZE, the latter technique often requires less method development effort, due to its huge separation power and its flexibility in selectivity tuning. In addition, applications on CZE instruments are very economical. No expensive LC columns are needed but very cheap fused silica capillaries, which, together with the electrolyte vials, require only a few milliliters of buffer solution.

Nowadays the use of capillary electrophoresis (CE) is one of the major trends in analytical chemistry, and the number of publications has increased exponentially in recent years, to several hundred a year. It is anticipated that capillary electrophoresis will spread in the analytical laboratories in forthcoming years, so at least its potential should be discussed in a book about techniques for environmental analysis.

17.1.2. Capillary electrophoresis and environmental analysis

Initially, CE was applied mainly to the field of biochemical analyses, but in recent years its applicability has been demonstrated in all fields of chemical analysis [9,10] including the analysis and purity determination of industrial products [11–13] and the separation of pollutants [14–20]. Despite the impressive CE separations shown at that time, it was concluded in 1990 that CE would not be suitable for environmental analysis unless special injection tricks were to become available [21]. Commercial CE instruments equipped with UV absorbance detectors are able to detect quantities as low as 200 fg (!) and can compete in this regard with the best GC systems. Unfortunately, the injection volumes which can be tolerated in CE, without decreasing the separation performance, are

typically a few nanoliters only, which implies a very poor sensitivity as expressed in sample concentration terms. The levels attainable by CE (several hundred ppb) are only found occasionally in environmental samples. Actually, governmental regulations require the determination of lower and lower levels of pollutants in environmental samples (for example, in the EC drinking water directive, that the concentration of any pesticide should be lower than 0.1 ppb) so the concentration sensitivity of commercial CE instruments is far from ideal. Some of these considerations were also valid for the application of capillary GC in environmental analysis. In practice, off-line extraction and concentration techniques are being used to increase the overall concentration sensitivity and, in recent years, special injection techniques have been successfully developed which allow the injection of up to 1000-fold sample volumes, or even entire LC fractions, onto the GC column [22]. In addition, special injection modes have been developed for CE, which allow the introduction of sample volumes as big as the entire volume of the separation capillary [23–25]. A simple clean-up step, followed by a large-volume injection and a high efficiency separation in a CZE system, should be adequate to fulfill the requirements of real-life environmental applications. The determination of polar and acidic or basic pollutants (e.g. modern pesticides in aqueous samples) would be a particularly interesting application.

17.1.3. Scope of this chapter

This chapter assumes some basic knowledge of CE theory and practice. The reader who does not yet have practical and theoretical experience with CE is referred to the book by Li [5].

Section 17.2 describes the different CE modes that are currently available. We show that CE is just the family name and that different CE modes have their own principles and abbreviations. Section 17.3 gives a short description of the equipment involved, with special emphasis on the systems that are commercially available. Section 17.4 deals with the special injection tricks that are required for large-volume injection and trace enrichment in capillary zone electrophoresis (CZE). Section 17.5 is about environmental applications. It is shown that the determination of pollutants in real matrices by CE is still rather rare. Finally, conclusions and future trends are given in Section 17.6.

17.2. CE TECHNIQUES

17.2.1. Capillary zone electrophoresis

Most of the CE work so far has been done using the capillary zone electrophoresis (CZE) mode [5,7]. Analytes are separated on the basis of differences in their electrophoretic mobilities, which are related to their charge densities. The separation is carried out in a capillary filled with a continuous background electrolyte (buffer). The direction and the velocity of migration of the analytes are determined by both electrophoresis and electroosmosis (Fig. 17.1). The latter phenomenon originates from ionized silanol groups on the inner wall of the fused silica capillary. They attract positively charged buffer ions

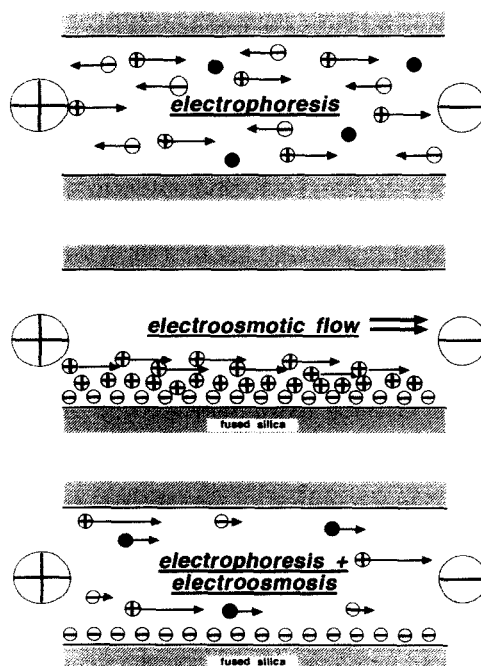


Fig. 17.1. Principles of electrophoresis and electroosmosis in a fused silica capillary.

which form an electrical double layer. The buffer ions in the mobile region of the double layer will migrate towards the cathode as soon as the voltage has been switched on and induce an electroosmotic flow of the entire liquid in the capillary. In an ordinary fused silica capillary at pH values above 5 or so, the electroosmotic flow will be higher than the electrophoretic migration velocity of most of the anionic analytes. Consequently, both cationic and anionic compounds will migrate in the direction of the cathode and can be separated within the same run. Contrary to LC, the (electroosmotic) flow might be influenced by sample constituents which interact with the capillary wall. Therefore, the reproducibility of migration times is not as good as the reproducibility of the electrophoretic mobilities (i.e. the mobilities after correction for the velocity of the electroosmotic flow). Also, regular integration outputs (mV versus time) are occasionally difficult to compare visually. The most important experimental parameters are the pH, the choice of the buffer and its concentration, and the applied voltage (or current). Its simplicity and similarity to elution chromatography have contributed to the popularity of CZE.

17.2.2. Electrokinetic capillary chromatography

Electrokinetic capillary chromatography (EKC) [5–8] is a sub-family of CE techniques, with the common feature that separations are based on the partitioning of the analytes between two phases having different velocities relative to each other. The most practiced mode, micellar electrokinetic capillary chromatography (MEKC or MECC) was

introduced in 1984 [26]. Usually the micelles are charged, and the uncharged analytes partition between the buffer phase (which migrates with the velocity of the electroosmotic flow) and the micellar phase, which is strongly retarded electrophoretically and acts as a pseudo-stationary phase (Fig. 17.2). There are several other options [5]: uncharged micelles with charged analytes, mixed micelles, etc. Other EKC modes are ion-exchange, cyclodextrin-modified and micro-emulsion electrokinetic capillary chromatography.

As compared to CZE, the EKC systems are more complicated because of the additional experimental parameters that have to be optimized. The peak capacity is restricted by the relatively narrow elution window which is determined by the velocities of the two phases. In addition, micellar systems are less stable than CZE systems because of the effect of the temperature on the equilibria involved. Despite these drawbacks, EKC has shown separations of neutral analytes with efficiencies as high as in CZE and, moreover, has demonstrated additional selectivity. This offers a nice alternative for pollutants which are not amenable to GC, and which suffer from the poor efficiency of LC.

17.2.3. Capillary isotachopheresis

Capillary isotachopheresis (CITP) [27] is a CE technique which is carried out in a discontinuous buffer system. The analytes are injected between the so-called leading and terminating electrolytes. A steady-state migration configuration is formed in which the analytes migrate as consecutive zones, which are not diluted by the background electrolyte as in CZE. The concentrating power of CITP may play an important role in trace analysis by CE in general. Contrary to the other CE modes and chromatographic systems, the isotachopherogram consists of a series of steps in which the step heights and the step lengths are indicative, respectively, of the identity and the quantity of the analytes. The current success of CZE will contribute to a revival of CITP, although the different nature of the latter might remain an important instinctive barrier for analytical chemists with a chromatographic background.

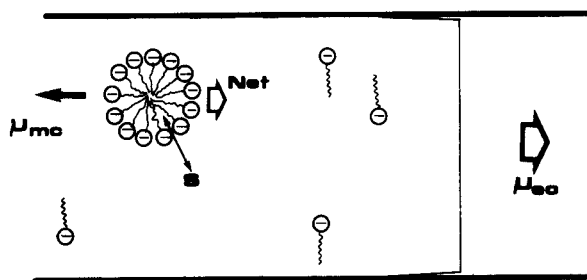


Fig. 17.2. Schematic representation of a system for micellar electrokinetic capillary chromatography (MEKC), showing anionic surfactant molecules, their micelles and the electroosmotic flow profile. S, an uncharged analyte, μ_{eo} , coefficient of electroosmotic flow, μ_{mc} , electrophoretic mobility of the micelle. Reproduced from [5] with permission of Elsevier Science Publishers.

17.2.4. Capillary gel electrophoresis and capillary isoelectric focusing

In capillary gel electrophoresis (CGE) [5], the capillary is filled with a cross-linked gel or with so-called entangled polymers. Separations of molecules are primarily based on differences in their size. Very high plate numbers (several millions) have been realized for biochemical analytes such as oligonucleotides and protein fragments which are normally separated by slab gel electrophoresis. Capillary isoelectric focusing (CIEF) offers separations based on differences in isoelectric points. Both techniques are almost exclusively applied in the biochemical field, and seem to be irrelevant for environmental applications.

17.2.5. Capillary electrochromatography

In capillary electrochromatography (CEC) [5,7], separations are based on partitioning between a mobile and a stationary phase. The stationary phase is either wall-coated in the capillary or the capillary is packed with LC material, having particle diameters as small as $1.5\text{ }\mu\text{m}$ [28]. The technique benefits from the flow profile of the electroosmotic "pump" which is essentially flat, and which leads to smaller plate heights than LC (parabolic flow profile). Both neutral and charged compounds can be separated using CEC. This technique is still not widely applied because of the lack of reproducible packed capillaries, which have been commercialized only recently.

17.3. INSTRUMENTATION FOR CAPILLARY ELECTROPHORESIS

A basic CE instrument consists of the following components: a high voltage power supply, electrodes, a safety compartment, buffer vials, the capillary, a conditioned capillary compartment, a detector, a data system and an injection device (Fig. 17.3). The following discussion refers to commercially available equipment only. Those readers who are interested in non-commercial options should refer to ref. 5.

- Power supply: typically able to provide constant voltages up to 30 kV and currents up to $100\text{ }\mu\text{A}$. The ambiguous title of this chapter can be understood, at least par-

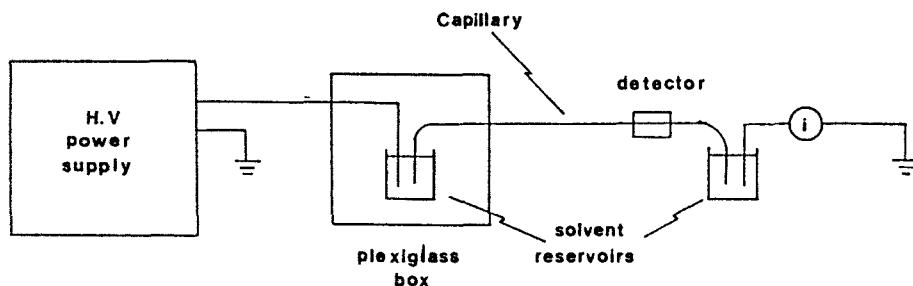


Fig. 17.3. Schematic representation of a system for capillary electrophoresis. Reproduced from [6] by courtesy of Marcel Dekker, Inc.

tially; the potential of CE is high indeed. Polarity switching can be either software-controlled, or carried out manually.

- Electrodes are usually platinum.
- All commercial instruments are equipped with safety interlocks which guarantee that the high voltage is switched off when the instrument is opened during an electrophoretic run and/or are provided with an automatic lock on the door(s), thereby preventing access to the electrode compartments.
- The buffer vials are typically as small as LC autosampler vials. Buffers are introduced into the capillary via a controlled pressure or vacuum system.
- The capillary is made from fused silica and typically is of 50 or 75 μm i.d. and has a length of 25–100 cm. Most of the separations so far have been carried out without a coating on the inner wall of the capillary. However, wall-coated capillaries have recently been introduced commercially and may be very useful reducing the electroosmotic flow or unintended interactions of the analytes with silanol groups at the fused silica surface.
- Most of the instruments are provided with a conditioned capillary compartment based on a fan, a thermostat and a fan, or a cartridge containing a thermostatted liquid.
- The instruments are equipped with a UV absorbance detector (either fixed or variable wavelength). The light path is limited to the inner diameter of the capillary which is, according to Beer's law, not very attractive for sensitive detection. Three companies offer a rapid scanning or diode array UV detector which yields a 3-D data matrix; the spectral information thus obtained can be helpful for identification purposes. One company offers a conventional and two companies offer laser-based fluorescence detectors.
- Dedicated CE data systems are still rare. Usually chromatographic integration software is applied. It should be stressed that these systems are not designed for CE. In CE, one has to quantitate by peak areas which have been corrected for the migration time (area/time) because of the fact that the analytes pass the detector with different velocities, as determined by their electrophoretic mobilities and the electroosmotic flow. In addition, the electroosmotic flow is not as constant as that provided by an LC constant flow pump, and, might be influenced by sample constituents. Consequently, electropherograms and the migration times of samples and external standards are occasionally difficult to compare visually. In practice, one would prefer to have the option of correcting the electropherogram for fluctuations in electroosmotic flow and to obtain an electrophoretic mobility axis as well as the time axis.
- The injection device: the sample volume which is consumed is typically a few nanoliters only so micro-vials can be used if evaporation is prevented by cooling and/or special vial caps. The sample is introduced into the capillary after replacement of the buffer vial at the high voltage end (cf. Fig. 17.3) by the sample vial. The sample can be introduced electrokinetically by switching on a specific voltage for a few seconds, while the capillary is inserted in the sample. The disadvantage of this injection method is the discrimination effect: components with higher mobilities are injected more than those with smaller mobilities. Another method uses siphoning; the sample vial with the inserted capillary is raised to a specific height for a few seconds, and the sample is injected due to the hydrostatic pressure created. Most of the instruments

offer the option of hydrodynamic injection via a controlled pressure or vacuum system at the capillary inlet or outlet, respectively. Usually a fixed pressure difference can be generated and applied for a specific time. Two instruments offer the possibility of both pressure and time control during injection. The precision of injection at the nanoliter level is often questioned. It is known by CE users that some commercially available instruments require the use of an internal standard in order to improve their quantitative reliability. However, some of the instruments do offer an injection precision better than 0.7% rel. SD at the 1–2 nl level. In the author's laboratory, two different CE instruments have been used in entirely different applications during the last 2–4 years, and internal standards have never been needed to improve the quantitation.

17.4. TRACE ENRICHMENT IN CAPILLARY ELECTROPHORESIS

17.4.1. Field-amplified injection techniques

The field-amplified injection techniques are based on the fact that the electrophoretic velocity of an ion depends linearly on the field strength, i.e. the applied voltage divided by the length of the capillary. When an analyte is dissolved in a sample matrix having a lower conductivity than the background electrolyte (buffer) in the capillary, then this analyte will experience locally an increased field strength and will migrate with a higher velocity (proportional to the ratio of the conductivities in the background electrolyte and in the sample matrix). When the analyte reaches the boundary between the sample matrix zone and the background electrolyte, it will slow down again and stack into a zone much shorter than the original sample zone, thus the analyte has been preconcentrated or focused on-column. This phenomenon is shown in Fig. 17.4. Samples are often dissolved in diluted buffer or in water. The original sample can be injected either hydrodynamically or electrokinetically. As can be seen in Fig. 17.4, cationic compounds are focused at the front of the sample zone, and anionic compounds at the back of the sample zone. For the sake of simplicity, the electroosmotic flow has been ignored so far. The total electroosmotic flow in the capillary will be composed of all local electroosmotic contributions; in the present case, the higher electroosmotic flow in the sample zone and the lower electroosmotic flow in the rest of the capillary. Of course, the influence of the sample zone is not significant when its length is very short compared to the total length of the capillary. However, when one intends to inject a larger volume of sample dissolved in diluted buffer, the following problem arises. A hydrostatic pressure is created by the mismatch between the local electroosmoses on both sides of the sample background electrolyte boundary. Thus, a laminar backflow is created which causes additional band-broadening [29] and restricts the maximum enrichment that can be obtained to a factor of about ten.

Figure 17.5 [30] shows what happens when the sample volume, having a matrix of ten-fold diluted buffer, is increased (hydrodynamic injection). The peak area increases linearly, but the peak heights show that at higher volumes the enrichment will be counterbalanced by the additional band-broadening. Normally, the detection limit of a pollutant can be as low as 200 ppb, using a UV absorbance detector operating at 200 nm [31]. In a CZE

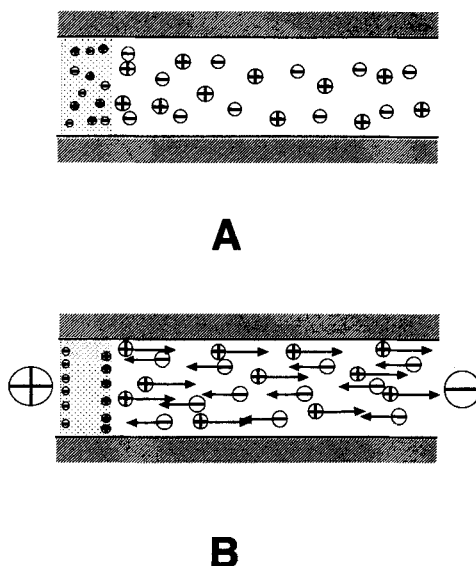


Fig. 17.4. Field-amplified injection. (A) A sample dissolved in diluted buffer, has been injected hydrodynamically. (B) The voltage has been switched on, the analytes within the sample zone experience a higher electric field and move with a high velocity to the boundary with the CZE buffer (focusing). The electroosmotic flow has been ignored for simplicity. Reproduced from [31] with permission of Elsevier Science Publishers.

system with a strong electroosmotic flow, which is normally the case, field-amplified injection can improve the detection limit towards 20 ppb. Fortunately, Chien and Burgi [23] showed that the enrichment factor obtainable by field amplification can also benefit from the electroosmotic flow. Their idea is elegant and simple; remove the relatively big sample matrix zone during or after the enrichment step, but before the start of the actual CZE separation, thereby avoiding the problem of the mismatch of local electroosmotic flows. This can be accomplished by pumping out the matrix zone, using the electroosmotic flow.

Figure 17.6 shows how to proceed for anionic analytes (the enrichment of cationic analytes demands the use of a modified fused silica capillary with a reversed surface charge and an electroosmotic flow in the direction of the anode [32–34]). A relatively large sample volume is injected hydrodynamically; next, trace enrichment by field amplification, and pumping out of the sample matrix by the electroosmotic flow, occur at reversed polarity of the applied electric field. Note that contrary to the normal situation (cf. below, in Fig. 17.1), the electrophoretic migration velocity in the sample zone will be much higher than the electroosmotic flow under these field-amplified conditions. Then, at a moment indicated by the value of the current (when the current has reached approx. 95% of its value when no sample zone is present), and the focused analyte zone has almost reached the beginning of the capillary, the polarity is switched back to its original position for the actual CZE separation. Careful monitoring of the current during the trace

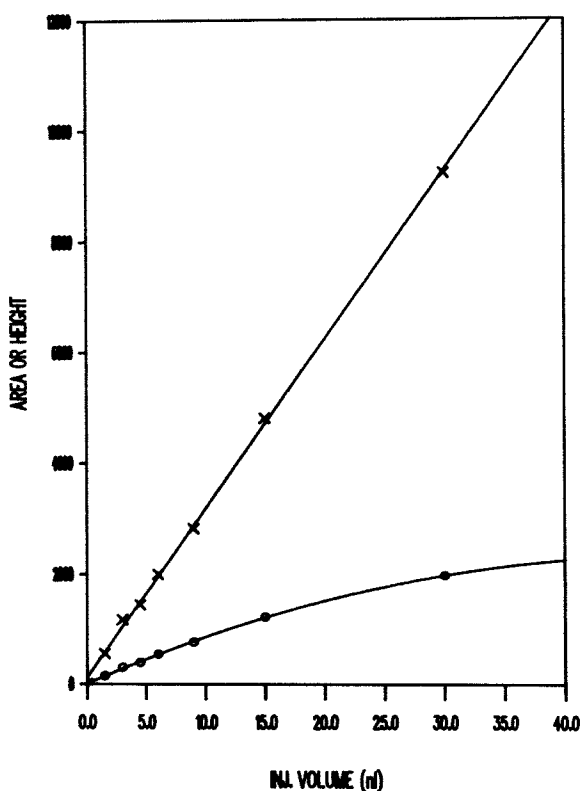


Fig. 17.5. Impact of the sample volume on the peak area (x) and peak height (O) (thus reflecting additional peak dispersion) under field-amplified injection conditions. Fused silica capillary 50 μm i.d. \times 72 cm; 40 mM ammonium acetate buffer (pH 4.8); separation at +25 kV. Analyte: *p*-aminobenzoic acid dissolved in tenfold diluted buffer solution. Reproduced from [30] with permission of Elsevier Science Publishers.

enrichment step at reversed polarity is essential to avoid loss of the enriched analytes into the buffer vial.

The maximum sample volume that can be preconcentrated in this way, without sample losses caused by initial backflush into the buffer vial, depends on both the electrophoretic mobility of the analyte and the overall magnitude of the electroosmotic flow [23]. Depending on these variables, the injection zones can be as long as one-third of the entire capillary length, or even more. It has been suggested that one can fill the entire capillary with the sample solution for convenience when large volumes cannot be hydrodynamically injected with sufficient instrumental accuracy and precision, thereby accepting the initial backflush and some sample loss. According to ref. 25, these large-volume injection techniques yield enrichment factors of more than 500, thus allowing environmental analysis at the low and sub-ppb level. The same technique can be extended to electrokinetic field-amplified injection by replacing the buffer vial by the sample vial during

the reversed polarity step. Of course, some discrimination will occur because of the additional electrokinetic injection, but enrichment factors can be as high as 1000.

From the real applications point of view, field-amplified injection requires samples to have a relatively low and reproducible conductivity. It is obvious that environmental sample matrices can be very diverse in nature, so in practice one has to filter the (aqueous) sample in order to avoid plugging and to exchange the original sample matrix for a matrix that meets the above requirements.

17.4.2. Solid phase extraction inside the capillary

Trace enrichment of (environmental) samples in CZE can be performed via solid phase extraction. A short plug of a reversed-phase type packing material can be incorporated at the beginning of the capillary [35] or a hydrophobic stationary phase can be covalently bonded to the inner wall of the capillary [14]. Because of the low amount of the wall-

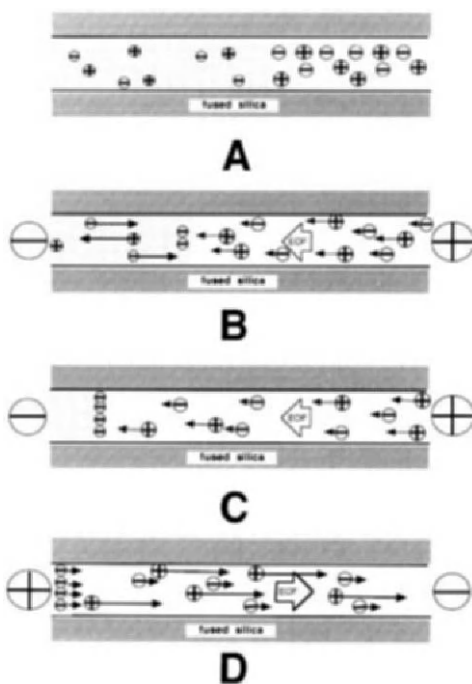


Fig. 17.6. Field-amplified injection of large sample volumes using sample matrix removal by the electroosmotic flow under reversed polarity conditions. (A) A very large volume of sample dissolved in diluted buffer has been injected hydrodynamically. (B,C) The voltage has been switched on and the original sample matrix is pushed back into the buffer vial by the electroosmotic flow. At the same time, anionic analytes in the sample matrix move with a very high velocity to the boundary with the CZE buffer (focusing). (D) The polarity is switched back to its normal position for CZE when the analyte zone has reached the beginning of the capillary. Reproduced from [31] with permission of Elsevier Science Publishers.

coated stationary phase, the breakthrough volumes and the capacity and hence the enrichment factors are rather limited (10–35-fold) [14]. The capillaries with the plug of reversed-phase material have a somewhat higher capacity and will show higher breakthrough volumes and enrichments (estimation: up to 100-fold). The disadvantages of in-column solid phase extraction should be mentioned. Unlike off-line solid phase extraction, one cannot introduce the raw environmental sample directly because of the risk of plugging the system and suffering severe contamination. In addition, one has to introduce a sample which is, at least partially, cleaned or extracted and should not contain a percentage of organic solvent. These types of capillaries are only available from a limited number of suppliers and are at least thirty times more expensive than a simple fused silica CZE capillary, which can be cut to the desired length and provided with a detection window, using a lighter, in your own laboratory.

17.4.3. On-line coupling of isotachopheresis and capillary zone electrophoresis

Isotachopheresis (cf. Section 2.3) is a technique by which sample zones of low concentration will be focused almost to the concentration level of the leading buffer, following the Kohlrausch regulating function [27]. From the point of view of trace analysis, on-line coupling of ITP and CZE is very attractive [36–38]. A typical example is shown in Fig. 17.7. In practice, one would prefer to inject a heart-cut from the analyte zone, focused and purified by ITP, into the CZE system. However, the focused analyte zone might be very short (a few micrometers) and its ITP migration time will be matrix-dependent, so the timing for the electrokinetic injection into the CZE part of the system will be very critical.

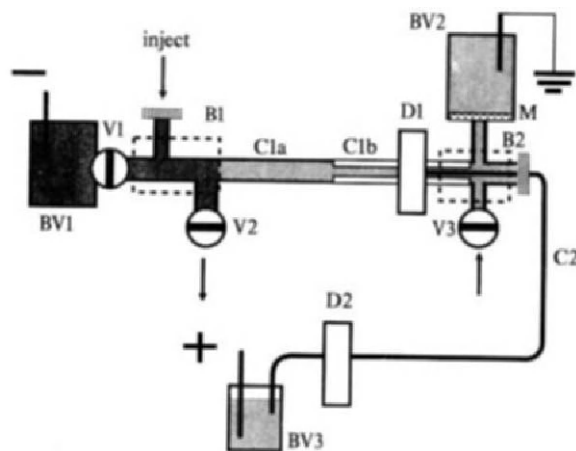


Fig. 17.7. Schematic diagram of an ITP-CZE system. V1, V2, V3, valves; D1, ITP UV absorbance detector; D2, CZE laser-induced fluorescence detector; M, membrane; BV1, terminating buffer vial; BV2, leading buffer vial; BV3, CZE buffer vial; B1, injection block; B2, ITP-CZE interface block; C1a, separation part of the ITP capillary; C1b, detection part of the ITP capillary; C2, CZE capillary. Reproduced from [38] with permission of Elsevier Science Publishers.

Reproducible transfer can be assured only when a larger zone from the ITP is injected into the CZE. Because of the split ratio at the ITP-CZE coupling point, only a part of the ITP zone (according to ref. 38, about 10%) is injected into the CZE system. Nevertheless, using a 10 μ l injection in the ITP-part, after on-line ITP-CZE, an enrichment factor of 1000 (as compared to CZE only) can be achieved, with a precision at the 0.5 ppb level of 3.7% rel. SD. Additional selectivity can be incorporated in the ITP part by proper selection of the leading and terminating electrolytes. Certainly, ITP-CZE is an interesting option for environmental trace analysis. Very recently, it has been demonstrated that the rather complex experimental set-up shown in Fig. 17.7, can be replaced by a single-capillary system using a fully automated commercially available apparatus [50,51].

17.4.4. Transient isotachopheresis stage in capillary zone electrophoresis

In regular CZE practice, samples are being dissolved in the background electrolyte (buffer). It has been shown in Section 17.4.1, that it might be very attractive to dissolve the sample in diluted buffer or water. On the other hand, it might be disastrous when a sample is dissolved in a matrix of very high conductivity because the sample zone may broaden and the separation efficiency and the concentration sensitivity may decrease significantly. There is one exception to this rule; when the sample matrix contains an excess of an ion having an electrophoretic mobility higher than the analytes, and when the buffer contains an ion with an electrophoretic mobility smaller than the analytes. In such a case, the high mobility matrix ion can act as a leading ion, and the buffer ion as a terminating ion, similar to ITP (cf. Section 17.2.3). The excess of high mobility ions in the sample matrix will be forced through and create a relatively long zone having a concentration adapted to the background electrolyte, following the Kohlrausch regulating function. Similarly, the zone of analytes having a lower concentration will be compressed, so that trace enrichment will occur as in regular ITP.

Beckers and Everaerts described this situation as electrophoresis with "two leading ions" [39,40]. Unlike the situation in ITP, the background electrolyte is continuous and the ITP-like state will be realized only for a limited period of time (transient ITP), since the zone of high mobility ions will start to migrate following the normal CZE mechanism. The analytes which have a mobility relatively close to the high mobility matrix ion will benefit from a later moment at which the ITP mode changes into the CZE mode, i.e. they will be enriched more than the analytes of relatively low mobility.

An example of transient ITP in the absence of an electroosmotic flow (at low pH) is shown in Fig. 17.8A: a relatively large volume of a sample of *p*-toluenesulphonic acid dissolved in the background electrolyte (10 mM phosphate buffer, pH 2.5), shows severe band-broadening and a distorted peak shape. The same sample dissolved in water shows peak focusing due to field amplification (cf. Section 4.1). Dissolution in an excess of sodium chloride shows the highest enrichment factor, and an increase in migration time, because of the huge chloride zone which has been forced through. Further optimization of the sodium chloride concentration and the injection volume yields the enrichment as shown in Fig. 17.8B. The analyte migrates close to the chloride zone, and the detection limit has been lowered to 5×10^{-8} M which corresponds to an enrichment factor of about 200. The peak width has reduced considerably and had to be registered at a data sampling

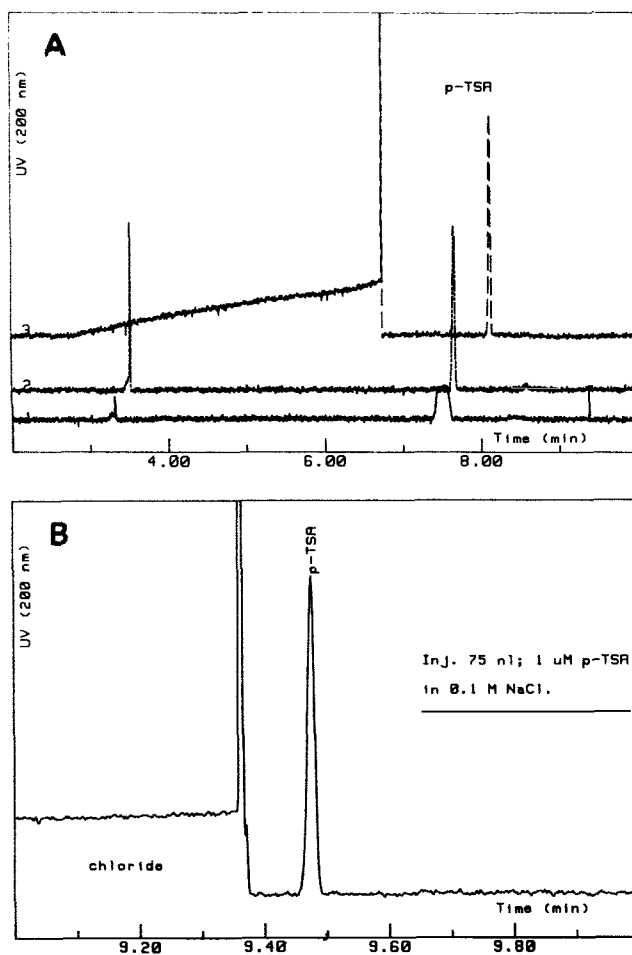


Fig. 17.8. (A) Electropherograms obtained after injection of 15 nl of *p*-toluenesulphonic acid (10^{-5} M) dissolved in (1) CZE buffer, (2) water and (3) 0.1 M sodium chloride solution. (B) Electropherogram after a transient ITP-CZE run, injection of 75 nl (10^{-6} M) in 0.1 M sodium chloride solution. Conditions: fused silica capillary $50\ \mu\text{m}$ i.d. \times 72 cm, CZE buffer 10 mM phosphate (pH 2.5) (electroosmotic flow negligible), separation at -25 kV (constant voltage mode).

frequency of 50 Hz (peak represents 3.7 million theoretical plates). The enrichment could be realized using an unmodified commercially available CE instrument.

The same principles can be applied to a CZE situation with the existence of a high electroosmotic flow. The major difference is that the sample matrix should contain an excess of a low mobility ion and the background electrolyte (buffer) should have an electrophoretic mobility higher than those of the analytes. In such a situation, the excess of low mobility ion will be forced through and its concentration will adapt and the analytes will be enriched and those having the smaller electrophoretic mobilities will undergo the ITP-

like process for a longer time. An example is shown in Fig. 17.9A. A 20 mM phosphate buffer (pH 6.3) was used as the background electrolyte and morpholinoethanesulphonate (MES) in excess as the matrix ion. Again, *p*-toluenesulphonic acid dissolved in buffer, water, or an excess of matrix ion, were compared and again the latter showed the highest enrichment factor and a relatively long migration time. Further optimization gives the result of Fig. 17.9B, which shows a peak (impurity) migrating close to the excess of MES, having 13 million theoretical plates, and a second peak having 3.6 million plates and an enrichment factor of about 100.

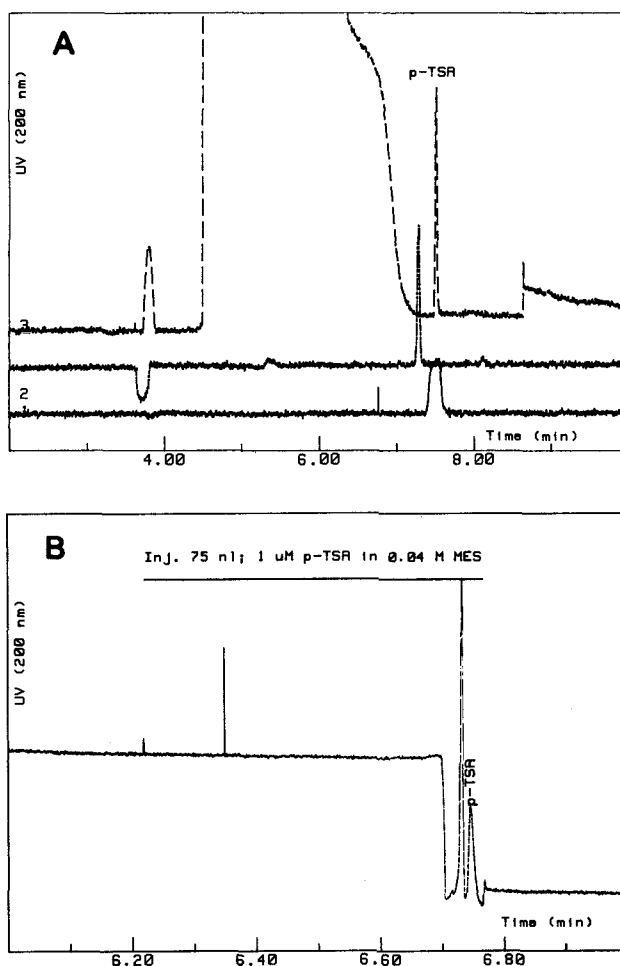


Fig. 17.9. (A) Electropherograms obtained after injection of 15 nl of *p*-toluenesulphonic acid (10^{-5} M) dissolved in (1) CZE buffer, (2) water and (3) 0.04 M morpholinoethanesulphonic acid solution. (B) Electropherogram after a transient ITP-CZE run, injection of 75 nl (10^{-6} M) in 0.04 M morpholinoethanesulphonic acid solution. Conditions: CZE buffer, 20 mM sodium phosphate (pH 6.3), separation at +25 kV; other conditions, see Fig. 17.8.

One should be aware of some disadvantages. As in ITP, it may occasionally be difficult to find a suitable combination of transient leading ion and terminating ion, the migration times of analytes in unknown samples may be hard to predict, and irreproducibility of the electroosmotic flow will give bad enrichment performance. In addition, it should be recalled that the analytes which migrate close to the matrix ion will take the maximum advantage of the ITP stage, so enrichment of analytes with a wide range of electrophoretic mobilities is not feasible. Despite these drawbacks, environmental trace analysis of sea water samples could very well be amenable to CZE with transient ITP, because of the presence of the high mobility saline matrix which can act as a leading ion in CZE separations without electroosmotic flow (cf. Fig. 17.8).

17.5. ENVIRONMENTAL APPLICATIONS

17.5.1. Separations of pollutants by capillary electrophoresis

Most of the environmental applications in CE are restricted to demonstrations of high resolution separations of specific pollutants and only a few refer to the determination of pollutants in real matrices. The reasons for this are obvious. CE is a relatively young technique, commercial instruments have been introduced only in recent years and are not yet widespread in the environmental analytical laboratories and, last but not least, the sensitivity has not been adequate for trace analysis (cf. Section 17.1).

Nevertheless, relevant separations have been demonstrated which might be converted into trace analytical methods provided that a focusing injection technique is used (cf. Section 17.4). The separations of pollutants include pesticides, phenols, phthalates, aromatic amines, nitro aromatics, surfactants and polycyclic aromatic hydrocarbons (PAHs). Cai and El Rassi [14] described the CZE separation of the triazine herbicides prometon and prometryne, after 10–35-fold trace enrichment in the capillary. They used two wall-coated octadecyl capillaries in series, one for trace enrichment via solid phase extraction and the second for the actual separation. Volumes of 100–120 nl of the aqueous samples containing 10 mM sodium phosphate buffer (pH 6.5) were preconcentrated and subsequently eluted and subjected to electrophoresis with 50% acetonitrile/phosphate buffer at 15 kV. The authors claim a detection limit of 100 ppb. The same authors described the CZE separation of paraquat and diquat herbicides [15]. A typical example is shown in Fig. 17.10. The use of a relatively high buffer concentration (0.1 M phosphate) was essential in this application in order to suppress the interactions of the cationic analytes with the negatively charged fused silica wall. The detection limits were 400–500 ppb. Nielen [12] described the CZE separation of phenylenediamine isomers using a 50 μ m i.d. uncoated fused silica capillary, or an apolar phase bonded to the capillary, with a 40 mM Tris/acetate buffer (pH 4.8) and at +25 kV. The detection limit of *o*- and *m*-phenylenediamines was approx. 500 ppb.

The same author described the CZE separation of phenoxyacid herbicides and some production related impurities [13]. A typical electropherogram is shown in Fig. 17.11. Baseline separation of the MCPP and 2,4-DP pair, and the MCPA and 2,4-D pair, was only achieved after careful pH optimization, using a 30–50 mM lithium acetate buffer (pH

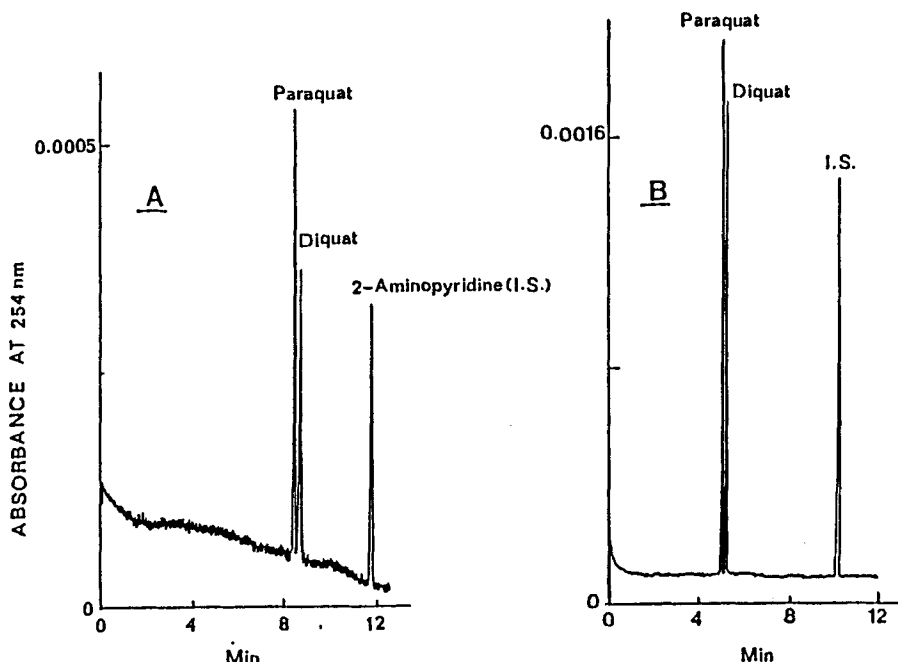


Fig. 17.10. Typical electropherograms illustrating the rapid separation of paraquat and diquat by CZE. Separation capillary, uncoated fused silica, $50\text{ }\mu\text{m}$ i.d. \times 80 cm; buffer, 0.1 M sodium phosphate (A, pH 3.5 and B, pH 7.0). Voltage +15 kV; detection by UV absorbance at 254 nm. Reproduced from [15] by courtesy of Marcel Dekker, Inc.

4.8). The potential of cyclodextrin-modified CZE for the chiral separation of racemic herbicides such as MCPP and 2,4-DP (Fig. 17.12) was described in the same paper. This could be very interesting for environmental studies, since only the *d*-2,4-DP and *d*-MCPP isomers are the biologically active components.

Yeo et al. [16] described the CZE separation of nine plant-growth regulators using a phosphate–borate buffer (pH 7.5), containing a cocktail of α -, β - and γ -cyclodextrins in order to enhance the selectivity. The electropherogram thus obtained is shown in Fig. 17.13. Desbène et al. [17] described the separation of linear C2–C12 alkylbenzene sulphonates by both CZE and MEKC. Baseline resolution was demonstrated using a CZE buffer system consisting of borate buffer (pH 9) in acetonitrile/water (3:7), a $50\text{ }\mu\text{m}$ i.d. fused silica capillary and a voltage of 30 kV.

The CZE separation of C9–C13 sodium alkylsulphates was shown by Nielsen [41]. The problem of the lack of a chromophore in the analyte molecules was solved using indirect UV detection. Local displacement of the UV absorbing background electrolyte yielded negative peaks with a separation performance similar to direct UV detection.

Terabe et al. [19,26] used MEKC for the separation of substituted phenolic analytes. Up to 16 phenols were separated within 20 min using a borate–phosphate buffer containing 50 mM SDS as the micelle-building surfactant. Plate numbers ranged from 200 000 to

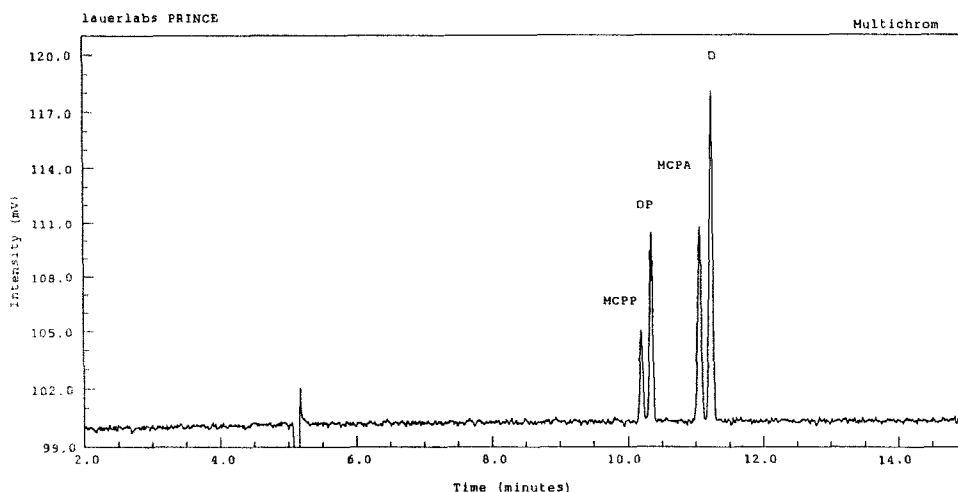


Fig. 17.11. Electropherogram of phenoxyacid herbicides by CZE. Conditions: fused silica capillary $50\ \mu\text{m}$ i.d. \times 79.5 cm; buffer, 50 mM lithium acetate buffer (pH 4.80); voltage +30 kV. Detection by UV absorbance at 200 nm (1 mAU/mV). Samples, 2-methyl-4-chlorophenoxypropionic acid (MCPP), 2,4-dichlorophenoxy propionic acid (2,4-DP), 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 2,4-dichlorophenoxyacetic acid (2,4-D), $((0.5\text{--}2) \times 10^{-4}\text{ M})$ dissolved in CZE buffer.

400 000 [26]. The quantitative aspects and reproducibility were investigated in another paper, using mono- to pentachlorophenols as model compounds in the ppm range [19].

The MEKC separation of eleven EPA priority phenols was shown by Ong et al. [42]. A typical electropherogram is shown in Fig. 17.14. The authors used a phosphate–borate

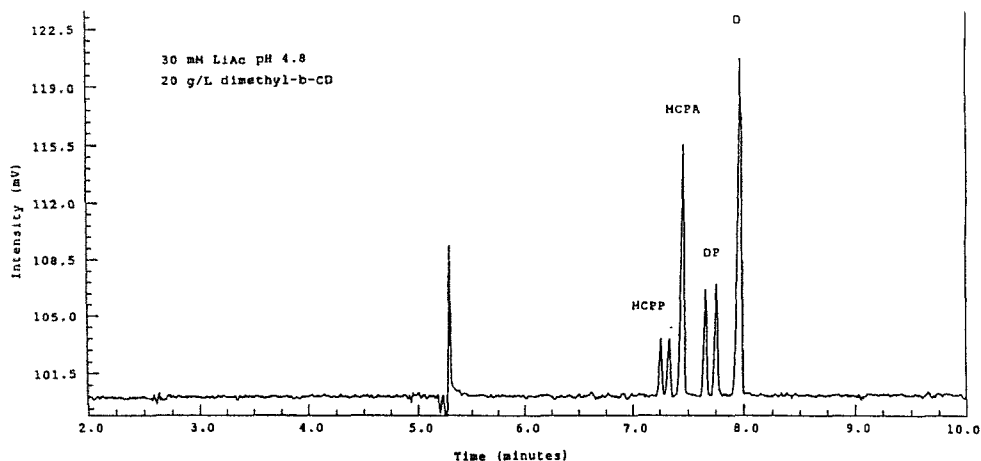


Fig. 17.12. Chiral separation of phenoxyacid herbicides by cyclodextrin-modified CZE. Buffer, 30 mM lithium acetate (pH 4.80) with 20 g/l heptakis-(2,6-di-*O*-methyl)- β -cyclodextrin. Other conditions, see Fig. 17.11.

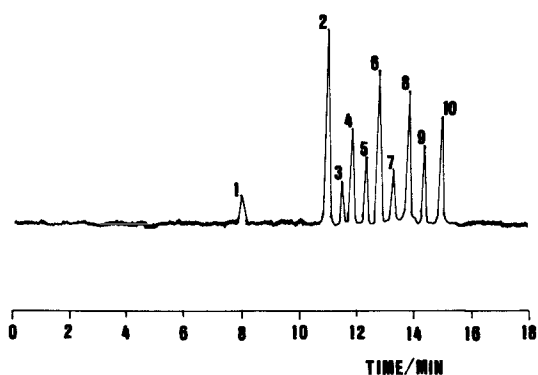


Fig. 17.13. Electropherogram of nine plant growth regulators by cyclodextrin-modified CZE. 1, 2,4-D; 2, gibberellic acid; 3, *p*-chlorophenoxyacetic acid; 4, indole-3-butyric acid; 5, 2,4,5-T; 6, β -naphthaleneacetic acid; 7, indole-3-propionic acid; 8, α -naphthaleneacetic acid; 9, indole-3-acetic acid; 10, unknown. Conditions: fused silica capillary 50 μ m i.d. \times 50 cm, buffer 0.05 M phosphate–0.1 M borate (pH 7.54) with 7.5 mM α -, 1.5 mM β - and 1.0 mM γ -cyclodextrin; voltage +15 kV; UV detection 196–210 nm. Reproduced from [16] with permission of Elsevier Science Publishers.

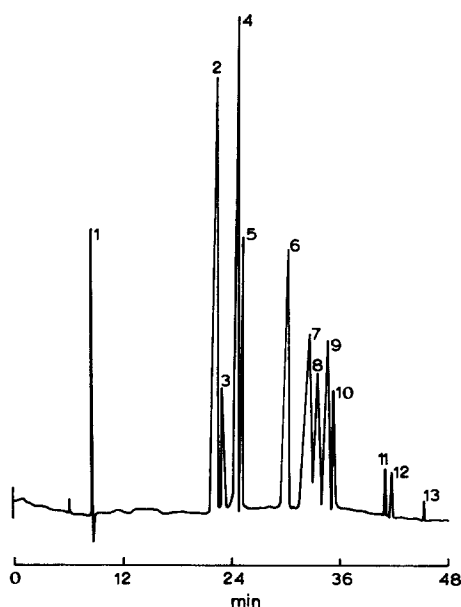


Fig. 17.14. Electropherogram of eleven phenols by micellar electrokinetic capillary chromatography. 1, methanol; 2, 4-nitrophenol; 3, 2,4-dichlorophenol; 4, 2-nitrophenol; 5, 2-chlorophenol; 6, 2,4,6-trichlorophenol; 7, pentachlorophenol; 8, 2-methyl-4,6-dinitrophenol; 9, 2,4-dinitrophenol; 10, phenol; 11, 4-chloro-3-methylphenol; 12, 2,4-dimethylphenol; 13, Sudan 3. Conditions: 180 μ m i.d. \times 1 m fused silica capillary; buffer 5 mM phosphate–10 mM borate (pH 6.6), containing 50 mM SDS; voltage +10 kV; UV detection at 254 nm. Reproduced from [42] with permission of Elsevier Science Publishers.

buffer (pH 6.6) containing 50 mM SDS, a fused silica capillary with a relatively large inner diameter (180 μm) and 10 kV. Undoubtedly, the performance of this separation can be further improved when a 50 μm i.d. capillary is applied (less Joule heating and less peak dispersion) at 20 or 25 kV.

The MEKC separation of nitroaromatics, including positional isomers, was shown by Yik et al. [43]. They used a phosphate–borate buffer (pH 7) and 30 mM SDS, and obtained baseline separation within 16 min. Ong et al. [44] described the MEKC separation of five EPA priority phthalate esters, using a phosphate–borate buffer (pH 6) and 10 mM SDS.

The MEKC separation of herbicides, featuring diode array UV absorbance detection, was shown by Wu et al. [20]. The optimized buffer system contained phosphate buffer, 100 mM SDS, 3 mM Brij-35[®] and 10% methanol. The separation was performed in a 50 μm i.d. fused silica capillary at 15 kV. The three-dimensional electropherogram thus obtained is shown in Fig. 17.15.

Walbroehl and Jorgenson [18] demonstrated CZE of PAHs using background electrolytes containing up to 50% acetonitrile and 25 mM tetrahexylammonium perchlorate. Of course normally PAHs are uncharged and are not amenable to CZE; in this particular study, however, solvophobic association of the PAHs with the tetrahexylammonium ions yielded cationic PAHs which could be successfully separated in the CZE mode. Other authors used cyclodextrin-modified MEKC for the separation of the same type of compounds [45,46].

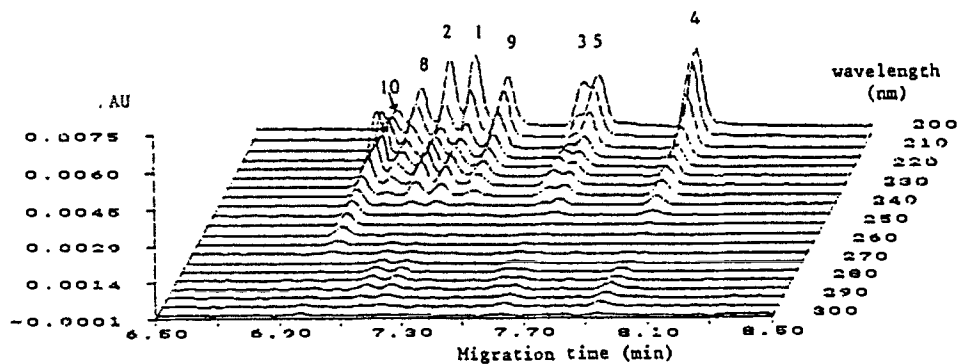


Fig. 17.15. Three-dimensional electropherograms of herbicides by micellar electrokinetic capillary chromatography and a fast-scanning UV absorbance detector. 1, 2,4-dichlorophenoxy propionic acid (2,4-DP); 2, 2-methyl-4-chlorophenoxypropionic acid (MCP); 3, 2-methyl-4-chlorophenoxyacetic acid (MCPA); 4, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); 5, 2,4,5-trichlorophenoxypropionic acid (2,4,5-TP); 8, dicamba; 9, 2,3,6-trichlorobenzoic acid (2,3,6-TB); 10, bentazone. Conditions: fused silica capillary 50 μm i.d. \times 44 cm; buffer 20 mM phosphate containing 100 mM SDS, 3 mM Brij-35[®] and 10% methanol; voltage +15 kV. Reproduced from [20] with permission of Friedr. Vieweg & Sohn Verlagsgesellschaft mbH.

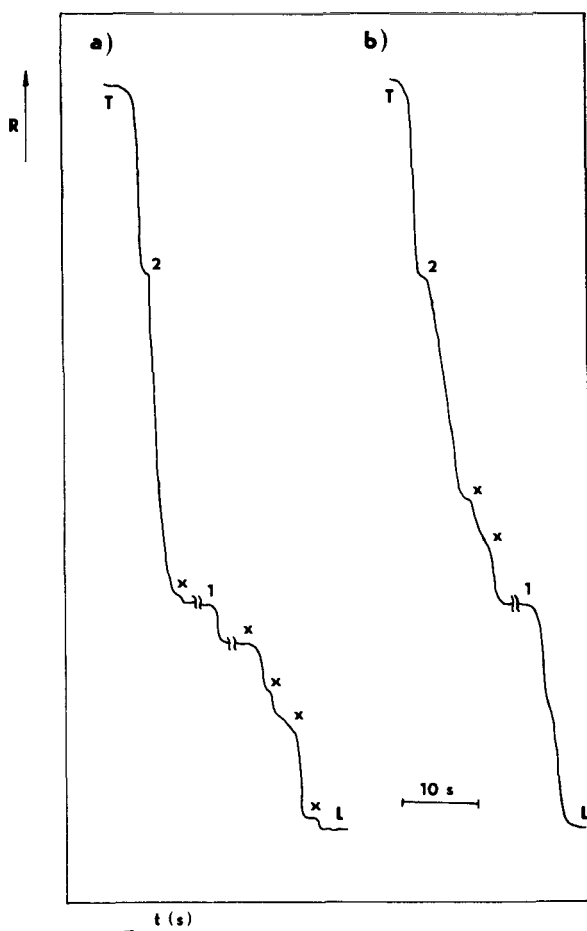


Fig. 17.16. Isotachopherograms of the determination of alphasmethrine in (a) water (25 ppb) and (b) soil (4 ppm). 1, phosphate; 2, *cis*-dichlorochrysanthemic acid (hydrolysis product of alphasmethrine). Capillary 300 μm i.d. \times 15 cm; L, leading electrolyte 10 mM HCl/creatinine/0.05% PVA (pH 4.80); T, terminating electrolyte 5 mM morpholinoethanesulphonic acid; detection by conductivity (R, resistivity); applied current, 30 μA ; injection volume, 30 μl . Reproduced from [47] with permission of Elsevier Science Publishers.

17.5.2. Determination of pollutants in real matrices

Dombek and Stransky [47] described the determination of the insecticides alphasmethrine and cypermethrine in water and soil samples by CITP (cf. Section 17.2.3). The samples were extracted with suitable organic solvents, which were then evaporated to dryness. The samples were then hydrolyzed in sealed ampoules at pH 12 and 50°C during 24 h. The degradation products thus obtained (*cis*- and *trans*-dichlorochrysanthemic ac-

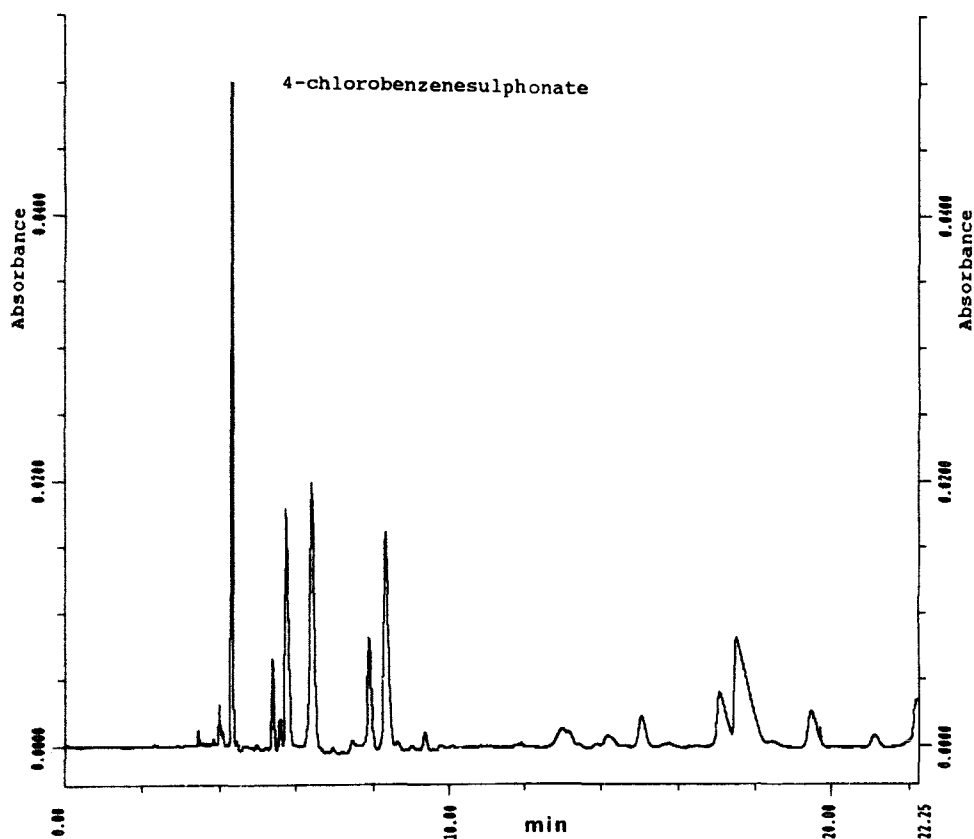


Fig. 17.17. Electropherogram of the leachate from a hazardous waste site by CZE. Conditions: fused silica capillary $50\ \mu\text{m}$ i.d. \times 57 cm; buffer 50 mM borate (pH 8.3); voltage +30 kV; UV detection at 214 nm. The highest peak is 4-chlorobenzenesulphonate. Reproduced from [48] with permission of Elsevier Science Publishers.

ids) were analyzed in an ITP system containing chloride as the leading electrode and morpholinoethanesulphonic acid as the terminating electrolyte. The isotachopherograms for water (25 ppb) and soil (4 ppm) samples are shown in Fig. 17.16. Recoveries ranged from 76 to 98% for surface water (50 ppb to 25 ppm) and from 60 to 88% for soil samples (0.5–50 ppm).

Brumley [48] separated eight aromatic sulphonic acids by CZE and applied the method to a leachate sample from a hazardous waste site. Aqueous leachate samples were concentrated by freeze-drying during 1–3 days, then the residue was redissolved in methanol and inorganic salts were precipitated by adding an equal volume of acetone. Finally, the filtered residue was evaporated and redissolved in methanol. An electropherogram of the leachate sample was obtained using a borate buffer (pH 8.3) and is shown in Fig. 17.17. The presence of 4-chlorobenzenesulphonate was confirmed by CZE coupled on-line with coaxial-flow FAB mass spectrometry.

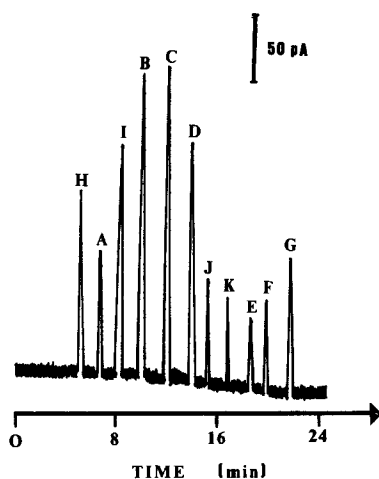


Fig. 17.18. Electropherogram of an industrial waste water sample with (A) a 2-chlorophenol concentration of 50 ppb by CZE. B, 2,4-Dichlorophenol; C, 2,6-dichlorophenol; D, *o*-phenylphenol; E, 2,3,4,6-tetrachlorophenol; F, 2-methoxy-4,5,6-trichlorophenol (4,5,6-trichloroguaiacol); G, pentachlorophenol; H, phenol; I, 4-chlorophenol; J, 1,2-dihydroxybenzene (catechol); K, 2,4,6-trichlorophenol. Conditions: 25 μm i.d. \times 65 cm fused silica capillary; buffer 45 mM orthophosphate–15 mM borate (pH 8.0); CZE at +20 kV; electrochemical detection using carbon fibers at +1.4 V versus SCE. Reproduced from [49] with permission of Elsevier Science Publishers.

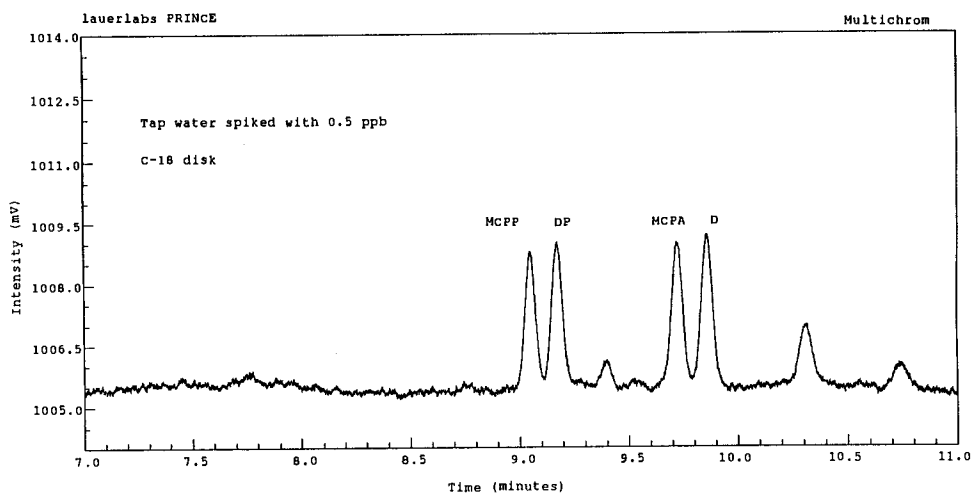


Fig. 17.19. Electropherogram of the trace determination of phenoxyacid herbicides in drinking water by CZE using field-amplified large-volume injection. D, 2,4-dichlorophenoxy acetic acid; other abbreviations as in Fig. 17.15. Conditions: fused silica capillary 50 μm i.d. \times 76 cm; CZE buffer 50 mM lithium acetate (pH 4.8); UV detection at 200 nm. Injection volume, about one-third of the volume of the CZE capillary. Trace enrichment by field amplification at –5 kV, followed by CZE at +30 kV. Sample of drinking-water spiked at the 0.5 ppb level and extracted and preconcentrated (20 \times) using C-18 Empore extraction disks. Elution with 50 μM lithium acetate buffer–acetonitrile (1:1). Reproduced from [31] with permission of Elsevier Science Publishers.

The analysis of industrial waste-water was demonstrated by Gaitonde and Pathak [49]. The waste-water samples were extracted with chloroform/diethyl ether and the organic layer was simply diluted with phosphate-borate buffer (pH 8). CZE was performed at 20 kV in a 25 μm i.d. fused silica capillary equipped with a laboratory-constructed electrochemical detector with 10 μm carbon fibres. Detection was done amperometrically at +1.4 V versus SCE. The electropherogram of a waste-water sample is shown in Fig. 17.18. Note that the 2-chlorophenol peak corresponds to 50 ppb.

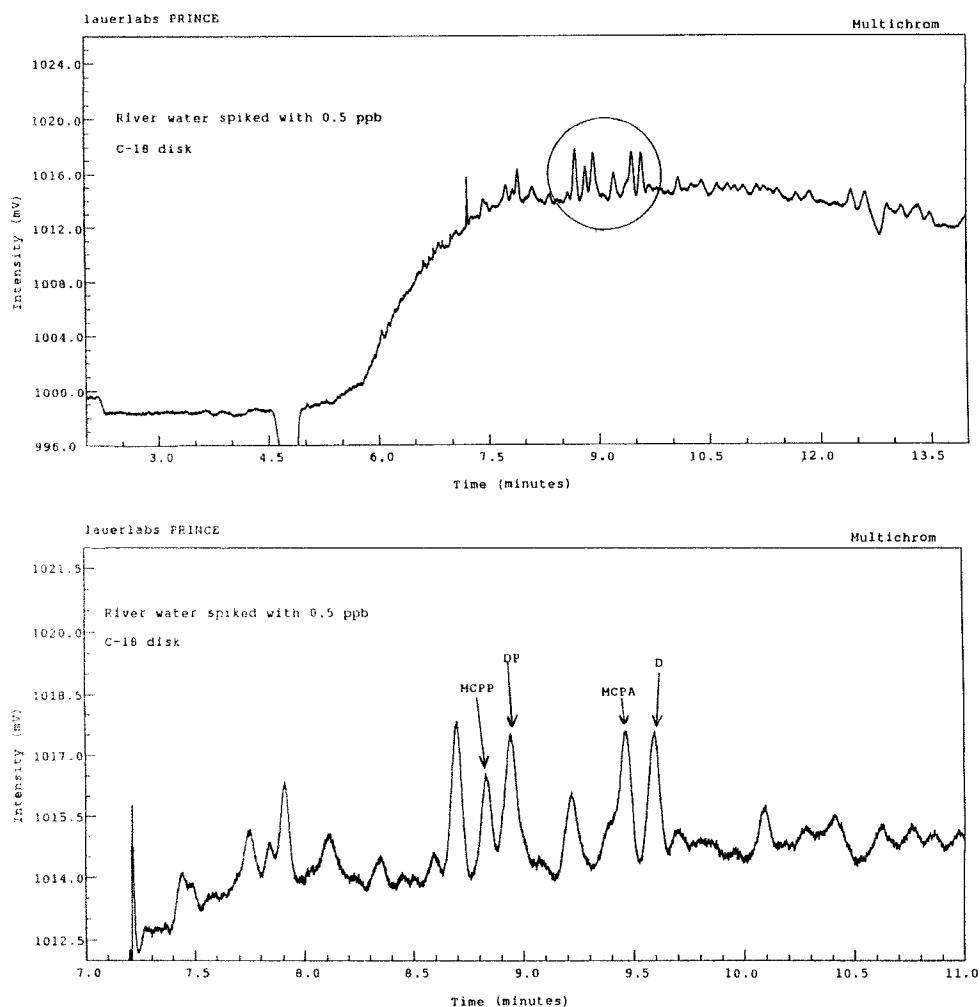


Fig. 17.20. Electropherogram and expanded view of the trace determination of phenoxyacid herbicides in river-water by CZE using field-amplified large-volume injection. Sample of river-water spiked at the 0.5 ppb level. Other conditions, see Fig. 17.19. Reproduced from [31] with permission of Elsevier Science Publishers.

Nielsen [31] described the determination of phenoxyacid herbicides in drinking-water and river-water samples by CZE with field-amplified injection (cf. Section 17.4.1). Sample volumes of 20–40 ml, spiked at the 0.5 ppb level, were acidified to pH 1 and pre-concentrated 10- or 20-fold by solid-phase extraction using C-8 and C-18 Empore extraction disks. The disks were eluted with 2 ml of 50 μ M lithium acetate buffer (pH 4.8) in acetonitrile/water (1:1), directly into the autosampler vials of the CZE apparatus. Due to the field-amplified injection, samples were enriched 200-fold in the CZE capillary and finally separated in a 50 mM lithium acetate buffer, and detected by UV absorbance at 200 nm. The recoveries in drinking-water were 98–103% and the detection limit was 50 ppt, which meets the stringent residue requirements of the EC drinking-water directive. The electropherogram is shown in Fig. 17.19. Although the river-water sample contained much humic material and insoluble matter, the recoveries were still 69–81% at the 0.5 ppb level. From Fig. 17.20, it is evident that a lot of anionic matrix components have been co-extracted. Nevertheless, the herbicides are well separated and can be quantitated. Bentazon could be determined in river-water samples in a similar way after the addition of α -cyclodextrin to the CZE buffer, which provided the additional selectivity required to separate bentazon from the phenoxyacids.

17.6. CONCLUSION AND FUTURE DEVELOPMENTS

Capillary electrophoresis (CE) represents a family of separation modes which can provide high resolution separations in short analysis times. Instruments have become commercially available and are similar in price to, or slightly more expensive than, LC systems. At least some of the manufacturers build instruments with good quantitative injection precision and accuracy (without the need for an internal standard), thermostatted capillary compartments, and variable wavelength UV absorbance detectors with excellent signal-to-noise characteristics. Compared to chromatographic separation systems, CE is more economical; with no expensive columns, only small volumes of buffer solutions, less maintenance and less method-development effort.

CE is very well suited for those analytes that are not amenable to GC or when existing LC methods do not offer sufficient separation power. Many impressive CE separations, including the separation of pollutants, have been demonstrated in the last few years. Due to the recently developed focusing injection techniques, CE has become competitive in trace analysis and the door has been opened to environmental applications in real matrices. Thus the potential of CE is very high indeed!

It can be predicted that a wider range of CE detectors will be offered by the instrument manufacturers. Current UV absorbance detectors were not specifically designed for CE, but are modified LC detectors. These detectors might be replaced by sophisticated detectors based on fibre-optic technology. More laser-based fluorescence detectors will be offered, and the high stability inexpensive diode lasers (with frequency doubling) might be incorporated in CE instruments at a later stage. Conductivity detection and electrochemical detection will be commercialized as well. The most interesting instrumental development, however, would be the introduction of a bench-top CE-MS with an atmospheric pressure electrospray/ion-spray source.

It is to be expected that CE instruments will be introduced in more and more analytical laboratories and the number of applications will continue to grow exponentially. Most probably, the nature of these papers will change gradually from "just showing a particular separation", towards applications in real environmental matrices. However, despite the excellent prospects for CE, many potential CE users may hesitate for the next few years, simply because the official methods do not tend to change quickly to incorporate new techniques.

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